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Arsenic Characterization in Soil and Arsenic Effects on Canola Growth.

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ARSENIC CHARACTERIZATION IN SOIL AND ARSENIC EFFECTS ON CANOLA GROWTH

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ABSTRACT

Reactions of soil arsenic with arsenic addition and the effects of soil arsenic on canola were studied because of a lack of information on this subject. The reactions of different pools of soil arsenic to arsenic addition were studied. The effects of soil arsenic and arsenic form and concentration in solution on canola growth and nutrient uptake were also investigated and an attempt to model arsenic uptake with a mechanistic computer model was made.

In a solution study, rate of inorganic arsenic did not appear to effect arsenic accumulation in roots and shoots of canola. However, shoot and root arsenic concentrations increased with organic arsenic rates. Arsenic to accumulated in the plant roots in both inorganic and organic treatments. Shoot dry weights were reduced when exposed to organic arsenic forms. Root length and dry weight were affected by all forms of arsenic. Shoot calcium and phosphorus levels increased while shoot zinc decreased with increasing arsenic rate.

In a soil study, soil solution arsenic increased curvilinearly, while resin-exchangeable solid-phase arsenic approached a maximum with arsenic addition. Initial solution arsenic concentration and DTPA-extractable manganese were correlated with the change of solution arsenic concentration due to arsenic addition. The relation between total diffusible and solution arsenic was described with nonlinear regression and was different for each soil.

In a growth chamber study, canola was sensitive to soil arsenic. A mechanistic computer model was used to predict arsenic uptake by canola. Using this model, root

growth rate and root radius were found to have the most influence on arsenic uptake. Plant arsenic levels increased significantly with increasing arsenic rate. However, arsenic tended to remain in the plant roots.

This study indicates that canola is sensitive to arsenic and that the form and concentration of arsenic affect toxicity. Furthermore, arsenic addition causes solution As to increase curvilinearly while resin-exchangeable solid-phase arsenic approaches a maximum. These changes in the soil As phases can lead to an increased bioavailability of the arsenic in the soil which can lead to increased uptake by plants that can be predicted using a mechanistic computer model.

INTRODUCTION

Arsenical compounds have been used in cotton production in Louisiana for nearly a century. Hence, some cotton-producing soils have elevated arsenic (As) concentrations. Little research has been done to define the effect of added arsenic on the arsenic phases in these soils. These soils are suitable for canola (*Brassica napus* L.) production, however, the elevated arsenic levels in these soils may prove to be a limitation to canola growth. This study was initiated to learn more about the effects of arsenic addition on the soil arsenic phases and on the growth of canola, a possible new crop for Louisiana.

Since organic and inorganic forms of arsenic have been used in cotton production, both of these forms may be present in the soil. The effects of these forms on canola need to be determined. If the different forms of arsenic have no effect on canola growth, then arsenic should not limit canola production. If, however, canola growth is affected by arsenic, then the extent of that effect must be determined.

A second area of needed research is to determine how arsenic addition affects the different arsenic phases in the soil. A great deal of research has been done on the effects of arsenic addition on total and extractable soil arsenic. However, little information is available on the effect arsenic addition has on the soil solution arsenic and the diffusible solid-phase arsenic phases in the soil. These phases can provide bioavailable arsenic to plant roots in the soil. The soil physical and chemical properties influencing the soil arsenic reactions also need to be defined.

A third area of research is to determine how soil arsenic levels affect canola growth and which soil or plant parameters have the most influence on arsenic uptake. Computer modeling can be used to determine the factors influencing arsenic uptake and may be used to predict arsenic uptake.

This dissertation includes a literature review consisting of general information on arsenic, arsenic reactions in the soil, and arsenic effects on plant growth. The literature review also contains general information on canola, ion movement in the soil, ion uptake by plants, and a review of the Barber-Cushman mechanistic model.

The first chapter of the dissertation consists of an experiment to determine how canola growth and nutrient uptake are affected by organic and inorganic arsenic in a solution culture. In Chapter 2, the changes in the soil arsenic phases with arsenic addition and how these changes affect arsenic bioavailability in the soil are investigated. The third chapter covers the effects of arsenic rate on canola growth and an attempt to model arsenic uptake using a mechanistic model.

This research should define a possible limitation to canola production on cotton-producing soils. It should also provide basic information on the influence of soil physical and chemical properties on various soil arsenic phases in the soil, and soil and plant parameters influencing arsenic uptake. Hence, this information can be used in the decision to produce arsenic-sensitive crops on cotton-producing soils.

Research Objectives

This research was initiated to determine the availability and soil reactions of arsenic added to soils as part of cotton production and the influence of this arsenic on a possible new crop to Louisiana. The specific objectives are to:

1. Determine the effects of organic and inorganic arsenic on growth and nutrient uptake of canola.
2. Determine the soil physical and chemical properties influencing arsenic movement in the soil.
3. Learn more about how arsenic addition affects the different arsenic phases in the soil.
4. Study the effect of arsenic addition to soils on the growth and arsenic uptake by canola.
5. Determine if a mechanistic computer model can accurately determine arsenic uptake.

The first objective was accomplished with an experiment using canola grown in a nutrient solution containing the different forms of arsenic. Results and conclusions of this experiment are presented in Chapter 1. The influence of the soil physical and chemical properties and arsenic addition on soil arsenic was studied in an experiment where arsenic was added to soils and different arsenic phases analyzed. These results are reported in Chapter 2. For the fourth and fifth objectives, a growth chamber study was used. Results from this study are presented in Chapter 3.

LITERATURE REVIEW

General Information about Arsenic

Arsenic is widely known for its lethal properties and has been and still is popular with fiction writers as a method for murder. In reality, only certain forms of arsenic are toxic and, in the past, arsenic has been used for medicinal purposes. Arsenic has been known since 2500 BC and used in medicine since 400 BC (Vallee et al., 1960). As early as 79 AD, local application of arsenic was used to treat ulcers (Kipling, 1977). In 1478, one of the first medical books published discussed prescription of arsenic. By the 1600's, arsenic was widely used as a medicine and appears in the Medical Dispensatory of 1608. Arsenic was used to treat the plague, tuberculosis ulcers, cancer, and skin ulcers. By the 19th century, arsenic was used to cure debility, anemia, epilepsy, asthma, and chronic skin diseases. Uses of arsenic as a medicine have declined greatly, however some countries still use orpiment (a sulphur - arsenic compound) (Kipling, 1977).

As arsenic was used as a medicine, it also has well documented toxic effects. There were many cases in Britain during the 1800's where arsenic was used as a poison to commit murder (Kipling, 1977). There are also many cases of accidental arsenic poisoning. Poisoning has occurred by handling playing cards, money, or wearing clothing that used arsenic based pigments. Exposure could also occur from pigmented paint, wallpaper, blinds, carpet, and linoleum (Kipling, 1977).

The element arsenic is a brittle, gray metalloid. It has three allotropic forms that can be yellow, black, and gray. Arsenic is a Group Vb. element in the Periodic Table and chemically resembles phosphorus. Because of this similarity, arsenic and phosphorus

can compete for chemical binding sites, which can adversely affect living organisms. Arsenic can be found in almost all natural environments. Over 245 minerals contain arsenic as a major component.

Arsenic has a variety of uses. These include agriculture, ceramics, glass, chemicals, and other miscellaneous uses. In agriculture, arsenic trioxide, As_2O_3, is the base material used to form insecticides, herbicides, fungicides, algicides, wood preservatives etc. Arsenic is used for these chemicals based on its toxic properties. Inorganic arsenic was used in cotton production from the late 1800's until the middle 1960's. During this period, calcium arsenate was the major arsenical used in cotton production. In the middle 1960's, use of inorganic arsenical herbicides declined due to the appearance of the organic arsenical chemicals. The carbon group attached to the arsenic ion facilitates movement of the herbicide through the leaf surface into the plant. Thus, the organic form of the chemical was more effective and could be used at lower application rates. Two common arsenic compounds used in cotton production in Louisiana are monosodium methane arsenate (MSMA) and disodium methanearsenate (DSMA). These compounds are applied as a directed spray to the leaf surface. However, arsenic returns to the soil through oxidation of dead plant tissue and overspray and can accumulate (Sandberg and Allen, 1975). This accumulated arsenic may prove harmful to sensitive plants grown in these areas.

Arsenic in Soils

Each soil's geology will determine its inherent arsenic content. Arsenic levels can accumulate to high concentrations where there is a history of prolonged arsenic use

(Adriano, 1986, Ori et al., 1993, Walsh and Keeny, 1975, Woolsen et al., 1971). However, in soils where no arsenic has been applied, levels average around 5 mg kg^{-1} and are rarely greater than 10 mg kg^{-1} (Shacklette and Boerngen, 1984, Selby, 1974, Vinogradov, 1959). Woolsen et al. (1971) compared arsenic levels in 58 surface soils with a history of arsenic use to arsenic levels in nearby soils with no history of arsenic use. The arsenic levels in the contaminated soils averaged 165 mg kg^{-1}, while the arsenic levels in the uncontaminated soils averaged only 13 mg kg^{-1}. A study comparing more than 450 samples from agricultural soils in Louisiana found an average of 23 mg As kg^{-1} with a range from below detectable limits to 73 mg kg^{-1} (Ori et al., 1993). Hence, soils with histories of cotton production average 4 to 5-fold what is normally expected in virgin soils. Soils near arsenic mineral deposits are exceptions. These soils can average 400 to 900 mg kg^{-1} (NRCC, 1978). Soils near sulphur deposits are also associated with high arsenic levels because of sulphur - arsenic compounds (Adriano, 1986).

Chemical Reactions of Arsenic

Arsenic is subject to chemical and/or microbial addition (reduction) or removal (oxidation) of electrons (Masscheyelan et al. 1991). The reduction or oxidation of arsenic depends on the amount of oxygen in the soil. In soils with oxygen present, arsenate (As V) is the predominate form of arsenic. As the oxygen level decreases and the soils become more reduced, arsenite (As III) becomes more prevalent. Arsenite is more soluble and, therefore, more mobile than arsenate. Arsenite is also more toxic. Arsenate and arsenite are the main forms of arsenic in the soil. These two forms of

arsenic are also susceptible to microbial methylation. It is this methylation that leads to two other common forms of arsenic in the soil, mono and dimethyl arsenic acid (Masscheleyan et al., 1991). These are the four common forms of arsenic found in the soil.

Masscheleyan et al. (1991) demonstrated the effect of redox potential and pH on arsenic solubility and form. They measured the form and concentration of arsenic in soils as the soils became more reduced. They found that arsenate reduced to arsenite as the redox potential decreased. The maximum total arsenic levels occurred at a redox potential between 0 and 100 millivolts (mV). These findings are supported by Bohn (1974). This would indicate that the most severe arsenic problems would be more likely to occur in soils that have been reduced for a period of time.

As stated earlier, arsenic and phosphorus belong to the same periodic family and will have similar reactions in the soil and with soil compounds. The competition for binding sites in soil was shown by Peryea (1991). Peryea (1991) added high rates of phosphorus to five soils that were contaminated with arsenic. He found that concentrations of dissolved arsenic increased as the amount of added phosphorus increased. This indicates that arsenic and phosphorus compete for binding sites in the soil.

Because phosphorus reacts with iron, aluminum, and calcium, arsenic is assumed to form insoluble compounds with these ions also. This assumption is supported by Fordham and Norrish (1974, 1979) who found that arsenic adsorption was controlled by

iron oxides present in the soil that they used. They also found that aluminum oxides would react with arsenic if they were present in large enough quantities.

The effect of iron and aluminum on arsenic adsorption is also supported by Jacobs et al. (1970) and Woolsen et al. (1971). Jacobs et al. (1970) found that sorption of arsenic increased as iron oxide content increased. They also found that the arsenic sorption capacity of the soil dropped if iron and aluminum components in soil were removed. This indicated that soils containing high amounts of iron and aluminum would adsorb more arsenic than soils with low amounts of iron and aluminum. Woolsen et al. (1971) found that iron - arsenate was the dominant form of arsenic in 58 surface soils with histories of arsenic application. When the amount of aluminum or calcium was high and the amount of iron was low, aluminum - arsenate or calcium - arsenate forms dominated. Masscheyelan et al. (1991) also found that arsenic content increased as soluble iron (ferrous iron) increased. As the Fe^{3+} in the iron - arsenate compound is reduced to Fe^{2+}, the iron - arsenate compounds dissolve and arsenic is released. This will lead to elevated levels of arsenic in the soil that can adversely affect the growth of plants. Manganese (Mn) arsenate complexes also form and, under oxidized conditions, $Mn_3(AsO_4)_2$ can control arsenic solubility (Hess and Blanchar, 1976; Sadiq et al., 1983). Hess and Blanchar (1976) found that manganese arsenate is more stable than iron, aluminum, lead, or calcium arsenate at low pH. Masscheleyn et al. (1991) reached a similar conclusion stating that arsenic solubility can be controlled by $Mn_3(AsO_4)_2$ as soil conditions become more reduced.

Evidence has also shown that arsenic adsorbs to clay particles in the soil (Hingston et al., 1971, Lumsdon et al. 1984). At pH levels typically found in the soil, Frost and Griffen (1977) showed that arsenate adsorption to kaolinite and montmorillinite peaked at a pH range from 4 to 6 and that arsenite adsorption on montmorillinite peaked at pH 7. Frost and Griffen (1977) also found that arsenite was adsorbed in smaller quantities than arsenate by both clay minerals. In this study, montmorillinite was shown to adsorb both arsenate and arsenite much more strongly than kaolinite. However, Goldberg and Glaubig (1988) found that adsorption of arsenate and arsenite by both clay minerals was similar. This difference between the two studies could be due to different extraction methods, or due to different methods of determination of arsenic.

In soils containing large amounts of iron, aluminum, manganese, or clay, arsenic toxicity may not be a problem. However, in loam or sandy loam soils where these soil factors may not be as influential, arsenic toxicity may occur.

Transport of Arsenic Within Soils

Movement of arsenic in the soil profile is strongly influenced by soil type and the soil chemical and physical properties. Isenne et al. (1973) reported that arsenic moved 46 cm into the soil after a high rate of arsenic was surface applied 14 years earlier. Concentrations of arsenic in the soil decreased as depth increased. The distance arsenic will move in a soil depends on the amount of arsenic applied, the soil type, chemical and physical properties of the soil, and the amount of water moving through the soil. If only small amounts of arsenic are applied, the arsenic can be adsorbed or complexed by

various soil fractions. Complexed or adsorbed forms of arsenic will not be able to move deeper into the soil profile. Soil type will likewise affect movement of arsenic. Soils high in clay minerals will retard arsenic movement much more than sandy soils (Frans et al., 1956, Steevens et al., 1972). Because sandy soils generally have a low clay content, less arsenic will be adsorbed and more will be able to move with water. The chemical properties of the soil will also affect arsenic movement. Soils high in iron, aluminum, manganese, or calcium can remove arsenic from the mobile phase, thus restricting its transport. The amount of water moving through the soil profile will also affect the distance arsenic moves. For example, Arnott and Leaf (1967) found no arsenic movement out of a column of soil when 1 l of water was passed through the soil. However, when 5 l of water were passed through the soil, arsenic appeared in the leachate.

Arsenic in Plants

In plants, arsenic toxicity symptoms include leaf wilting, purpling, and root discoloration. In addition, different plants have sometimes shown unique responses to arsenic. For example, rice plants have shown a decrease in tillering (Chino, 1981). It should be noted that the symptoms of arsenic poisoning are similar to those of phosphorus deficiency. It has been suggested that arsenic may be substituting for phosphorus in plant metabolism (Amburgey, 1967). While arsenic can substitute for phosphorus, it cannot duplicate phosphorus' function in the plant. If arsenic replaced phosphorus as a component of various compounds within the plant, the plant's metabolism would be affected and the plant would show phosphorus deficiency

symptoms. Hence, a soil test would show adequate levels of phosphorus while the plant would show deficiency symptoms. Such a situation could indicate high arsenic levels in the soil.

Plant Tolerances to Arsenic

Plants have shown varying tolerances to arsenic. Jacobs et al. (1970) studied the effect of arsenic levels on vegetables grown in sandy soil. They applied arsenic levels 45 to 720 kg As ha^{-1} and grew potatoes in 1967, snap beans, peas, and sweet corn in 1968, and peas in 1969. The researchers found that the potato yields in 1967 decreased at high rates of arsenic. Yields of snap beans and corn also decreased with increasing arsenic levels and no growth was found on the high arsenic soils. Crop tolerances were: potatoes > peas > sweet corn > snap beans. Liebig (1966) found similar crop tolerances. Jacobs et al. (1970) also studied the arsenic levels found in various portions of the potato and in the seeds and pods of the snap beans. Arsenic concentrations increased in the potato flesh and peel and in the snap bean seed and pod as arsenic application rates increased. In the potato tissue, arsenic levels in the peel were higher than in the flesh.

In general, bean crops, most of the legumes, and rice are sensitive to arsenic, while plants such as carrots, tomatoes, wheat and oats are tolerant (Adriano, 1986). The different responses of plants to arsenic could be due to different root systems, altered uptake mechanisms, or to different exudates being emitted by the roots. Exudates could complex with the arsenic, causing the toxicity to drop. On the other hand, exudates could reduce arsenate to arsenite and enhance the toxic effect.

Effects of Arsenic Form on Toxicity and Translocation

The form of arsenic also affects toxicity. Marin et al. (1991) studied the effect of four forms of arsenic [arsenate, arsenite, monomethyl arsenate (MMA), and dimethyl arsenate (DMA)] on the growth of rice. They found that the plants treated with arsenite and MMA were stunted. There was also strong indication that plants treated with arsenite were going to die. These indications were yellowing, stunted growth, and severe wilting. The relation between arsenic form and toxicity has also been reported by others (Deuel and Swoboda, 1972, Reed and Sturgis, 1936, Vandecaveye et al., 1936).

The form of arsenic has also been shown to affect movement in the plant. A study comparing DSMA and sodium arsenite showed that both compounds move in the plant, but DSMA was much more mobile (Rumberg et al., 1960). However, Rumberg et al. (1960) noted that toxicity symptoms occurred sooner in the arsenite - treated plants and may have affected transport. Other studies have shown that arsenic compounds move differently within a plant. Sachs and Michael (1971) examined root absorption of MSMA, cacodylic acid (an arsenical herbicide), arsenate, and arsenite. They found the concentration of arsenic in the roots to be in the order: arsenate > arsenite > MSMA > cacodylic acid. The concentration of arsenic in the shoots was in the order of arsenite > arsenate > MSMA > cacodylic acid. However, when they compared the ratio of shoot arsenic to root arsenic levels, cacodylic acid was found to be transported 5 to 10 times faster in the plant than the other three compounds.

The relation between arsenic form and transport in the plant is important in determining where that arsenic form locates in the plant. For example, an arsenic form that is less toxic to plants may be transported to the seed of a plant (Rumberg et al., 1960). This could lead to consumption of elevated levels of arsenic by animals.

General Information on Canola

Canola generally grows well on loam or sandy loam soils. These soils are also used for the majority of cotton production in Louisiana. Thus, canola may be well suited for double cropping with cotton. A possible limitation for canola production is the use of arsenic compounds in cotton production in Louisiana. Arsenic from agrichemicals used for cotton production may be present in elevated levels and affect canola growth. Little research has been conducted on the effect of arsenic on canola, however wild mustard (*Brassica kaber*), a relative of canola, has been shown to be sensitive to arsenic (U.S. EPA, 1975).

Canola was developed from rapeseed in the late 1960's to provide a high quality, edible vegetable oil after processing. One of the largest advantages of canola oil is its low concentration of saturated fat. Canola contains only 6% saturated fat, compared to 11% saturated fat in sunflower seed oil and 15% saturated fat in soybean oil (Shahidi, 1990). Because of its low fat content, demand for canola oil is growing as health consciousness increases. In addition, like sunflower seed, canola seed consists of about 40% oil compared to the 18% oil content of soybean seed (Shahidi, 1990). Canola meal contains protein (36-38%) comparable to sunflower (28%) and soybean meal (44%) (Shahidi, 1990).

For safe human consumption, canola oil must contain less than 2% erucic acid. Any rapeseed oil with more than 2% erucic acid is not considered canola oil and can only be used for industrial purposes. Rapeseed with >2% erucic acid is known as high erucic acid rapeseed. Canola can be referred to as low erucic acid rapeseed.

There are two types of canola grown in the United States. In regions where winters are severe, spring canola is grown and in milder regions, winter canola (Raymer et al., 1990). The requirements for winter canola are similar to those of winter wheat.

Movement of Ions in Soil

An important concept in understanding the relationship between ion uptake by plants and ions in the soil is that of ion mobility in the soil. This relationship involves the extent of ion movement through the soil to the plant root. In 1954, Bray developed a concept to describe the mobility of nutrients from the soil to the root. While this theory was developed for nutrients, it holds true for other ions in the soil. The concept divides ions in the soil into mobile and immobile groups. The mobile ions are those ions that are not typically adsorbed to the soil exchange surfaces and are soluble. Hence, these ions are readily available for plant uptake at the root surface and can diffuse through large distances in the soil. Nitrate-N and sulfate-S belong to this group of ions. The second group of ions, the immobile ions, are those ions whose mobility decreases with distance from the root. These ions are generally adsorbed to exchange sites on the surfaces of the soil solids and include the exchangeable cations and phosphorus. Arsenic would also fall into this group. The ions on exchange surfaces in the soil are in equilibrium with the ions in the soil solution. As the ions in the soil solution are depleted by absorption into the

plant root, ions on the colloid surfaces dissociate into the soil solution. As uptake continues, the colloid surfaces near the root become depleted. These surfaces then begin to compete with the root for ions moving through the soil solution and thus, ion mobility in the soil decreases.

Bray (1954) defined two root absorption zones in the soil because of the differences in ion mobility. The root system absorption zone encompasses the volume of soil occupied by the entire root system. It is from this zone that mobile ions are absorbed. The root surface absorption zone is the second zone. This zone encompasses the volume of soil directly adjacent to the root surface. Immobile nutrients are absorbed from this zone. This zone also exists for new roots moving into previously untapped soil.

Ion movement through the soil to roots is governed by three processes; mass flow, diffusion, and root interception (Barber, 1962). Root interception is a term that describes the direct contact between ions held on the soil colloid surface and the root. No movement of the ion is necessary. Since roots occupy only about 1% of the total soil volume, root interception is generally ignored as a major mechanism of ion movement to the root (Barber, 1984). Mass flow is the movement of ions to the root with the convective flow of water in the transpiration stream. Generally, this is the main mechanism by which mobile ions or ions in large quantities in the soil move to the root. The amount of ions moved to the root by this mechanism can be calculated by multiplying the soil solution concentration of the ion by the amount of water absorbed from the soil by the plant. The third mechanism, diffusion, is the kinetic movement of

ions (Brownian movement) along a concentration gradient. As roots take up ions at the root surface, the concentration of available ion in the soil solution at the root surface diminishes, causing a concentration gradient away from the root into the soil. Ions in the soil then move along this gradient from higher concentration to lower concentration in an attempt to reach equilibrium. Since the roots are continually absorbing ions from the soil solution, equilibrium between ions in the soil solution at the root surface and ions in the bulk soil is never established, thus, ions continually diffuse to the root. Fick's second law can be used to describe transient state diffusion such as plant root-soil applications. Fick's law is expressed as:

$$\delta C/\delta t = D\delta^2 C/\delta x \qquad (1)$$

where $\delta C/\delta \tau$ is the change in concentration with time at a fixed linear distance, D is the diffusivity of an ion in water, and x is the distance. While this equation works for set linear distances, plant roots provide a radial sink for absorbing ions. When a radial component, r, is substituted for the linear component, x, the equation becomes:

$$\delta C/\delta \tau = 1/r\ \delta/\delta r\ (rD\ \delta C/\delta r) \qquad (2)$$

with r representing the radial distance from the center of the root cylinder. This equation was developed for movement of ions through a uniform medium such as water. However, soil is not a uniform medium, thus diffusion can be influenced by the physical and chemical properties of the soil. These factors either singly or combined can reduce the diffusion coefficient of ions in soil compared to the same ions in water. Nye and Tinker (1977) took these factors into account when they developed an equation to describe ion movement in the soil. This equation was:

$$D_e = D_l \theta_v f / b \qquad (3)$$

In this equation, D_e is the effective diffusion coefficient of the ion in the soil, D_l is the diffusivity of the ion in water, θ_v is the volumetric water content of the soil, f is a factor accounting for the tortuosity and impedance of the diffusion pathway, and b is the buffer power of the soil for the ion of interest.

Walker and Barber (1962) provided evidence to support the theory of mass flow and diffusion. Using rubidium-86 and strontium-90 and autoradiography, they illustrated the processes of diffusion and mass flow. Barber (1962) summarized his findings by saying:

> "The process that has the greatest effect on the availability for a particular nutrient depends on the concentration of the nutrients in the water which moves toward the plant root as a result of water uptake by the root, on the amount of water uptake which dictates the flow rate of this water, and on the rate of uptake of the nutrients by the plant root."

In determining which process, mass flow or diffusion, is the dominant mechanism, Barber (1962) said that when the ions move to the root in quantities greater than the root can absorb, and hence, collect at the root surface, then mass flow is the dominant mechanism. For diffusion to be the dominant process, mass flow can only supply a small fraction of the plant uptake and a concentration gradient must be established due to root absorption of ions (Barber, 1962). While Barber's study was developed for nutrients, the principles he describes hold true for any bioavailable ion found in the soil. Barber (1962) concluded that diffusion was the main mechanism for phosphorus and potassium movement to plant roots. Because of the chemical similarity

between phosphorus and arsenic, diffusion may also be the main supply mechanism for arsenic.

Factors Affecting Diffusion

Several soil chemical and physical properties influence diffusion either directly or indirectly. These factors include the ion concentrations in the soil solution and on the solid phase, the pathway the ion must follow from the source to the sink (impedance or tortuosity factor), the bulk density, water content, clay content, and temperature of the soil, as well as the size of the diffusing ion. Either singly or together these factors can exert a large influence on the diffusion of an ion through the soil.

Ion Concentration

The ion phases in the soil that affect diffusion can be separated into two phases, that in soil solution, and that on the soil solid phase that can move into solution. These phases are used to determine the buffer power of the soil. This equation is:

$$b=\delta C_{sp}/\delta C_{l} \qquad (4)$$

where δC_{sp} represents the change in the diffusible solid phase ion concentration and δC_{l} represents the change in the soil solution concentration of the ion. As the buffer power of the soil decreases, it becomes more difficult for the diffusible solid phase to maintain the solution phase concentration over time, however there are more ions moving to the sink and the effective diffusion coefficient increases. Conversely, at a high buffer power, the diffusible solid phase can more readily maintain the solution concentration over time. However, fewer ions will be moving to the sink and thus, a lower effective diffusion coefficient results. Typically, the buffer power relationship is curvilinear as the solution

ion concentration increases, thus the slope must be determined by differentiation of the buffer power curve at the solution concentration of the ion. The buffer power is the variable "b" in the effective diffusion equation.

Impedance Factor

The impedance factor, or tortuosity factor, takes into account the pathway and the concentration gradient the ion must follow from the source to the sink. This factor may also account for the differences in the water viscosity near the charged surfaces in the soil (Nye and Tinker, 1977). However, this change in viscosity would only affect a small part of the total water content of the soil. Barraclough and Tinker (1981) empirically determined the tortuosity of soils as related to the water content. Using a bromine-nitrate ion-counterion system and an ion exchange paper to measure the effective diffusion coefficient and then back calculating to determine the tortuosity values, they found that their data fit the following relationship:

$$f=1.58\theta_v-0.17 \qquad (5)$$

where f is the impedance factor and θ_v represents the volumetric water content of the soil. This value is the variable "f" in the effective diffusion coefficient equation.

Bulk Density

In conjunction with the impedance factor, the bulk density of the soil also affects diffusion. As the bulk density of the soil increases, the pathway the ion must follow becomes straighter and thus, diffusion increases because the soil solids are closer together (Barraclough and Tinker, 1981). Contrary to this is the findings of Warncke and Barber (1972) who found that as bulk increased, diffusion also increased until the

bulk density reached 1.5 g cm^{-1} after which diffusion decreased. Barraclough and Tinker (1981) attributed this difference in findings to the fact that Warncke and Barber (1972) had held the gravimetric water content constant causing the volumetric water content to increase as the bulk density increased. Barraclough and Tinker (1981) went further in the explanation stating that the decrease in diffusion was probably due to the movement of water from macropores to micropores.

Water Content

The volumetric water content of the soil is very important to the diffusion of an ion from a source to a sink. Water in the soil provides the medium through which the ion travels. The amount of water in the soil directly determines the cross-sectional area of the diffusion pathway. Hence, diffusion through soil increases proportionately with water content (Mahtab et al.,1971). The soil water content also affects the tortuosity factor in the soil. As the water content of the soil increases, the water films extend out from the soil solids. When the films bridge the airspace between the solids, the diffusion pathway becomes shorter than if the ion must move through the water held closer to the soil solids. Since the diffusion pathway is shorter, the diffusion rate is faster compared to the diffusion rate in drier soils.

Clay Content

The clay content of the soil can also affect the diffusion of ions. Mahtab et al. (1971) found that increasing the clay content also increased the diffusion rate. Increasing the clay content causes the volumetric moisture level of the soil to rise, increasing the cross-sectional area available for diffusion. They also found that a

reduction in available water had less of an effect on diffusion in clay soil than in courser-textured soils. This is due to diffusion being dependent on total volumetric water, not available water. Because clay soils have higher total water contents than lighter soils, they can withstand more reduction in available water without greatly affecting diffusion rates. Sharma and Kalia (1985) found results similar to those of Mahtab et al. (1971). They also found that diffusion increased with soil surface area which would increase as the clay content increased.

Temperature

Temperature can also affect diffusion of ions. A study by Singra Rao and Datta (1983) showed a linear increase in phosphorus (P) diffusion as temperature was increased from 25°C to 30°C to 35°C. The Stokes-Einstein equation:

$$D=k_bT/(6\pi r_i n) \qquad (6)$$

is used to describe diffusion of ions in water. In this equation, k_b is the Boltzmann constant, T is the absolute temperature, r_i is the ionic radius of the ion, and n is the viscosity of water. Changing the absolute temperature 10°K will only change the diffusion value directly about 4%, however this change in temperature will cause a large change in the viscosity of water resulting in a large change in the diffusion value (Weast, 1982).

Size of the Diffusing Ion

The size of the diffusing ion can also affect diffusion. If the molecule is within an order of magnitude of the pore diameter, diffusion of the ion can be reduced (Nye and Tinker, 1977). Two reasons exist for this reduction in diffusion. The first is that the

cross-sectional area of the diffusion pathway will be reduced. The second is that the Stokes Law applies to particles moving in an "infinite" medium. The viscosity of the medium will increase near the pore wall. This increase will result in a drag effect being felt by the ion. The combination of these two factors has been shown to slow diffusion (Renkin, 1954; Barraclough, 1976; Willams et al., 1966,1967).

Ion Uptake by Plant Roots

Once the ion has moved through the soil to the plant root surface, it must be absorbed into the plant. Two transport mechanisms exist for this uptake, active and passive transport. As active transport connotates, metabolic energy in the form of adenosine triphosphate (ATP) is expended to move an ion across the cell membrane against an electrochemical potential. Active transport is a commonly accepted occurrence (if respiration is inhibited, ion uptake stops). However, the mechanism for active transport is not well understood. Several theories exist as to how active transport occurs. In 1935, Osterhout suggested the involvement of a "carrier" molecule. This carrier can bind selectively to certain ions and transport them across the membrane. Thus, the cell could selectively control the ion movement into the cell. These carriers are generally small proteins that bind the ion on the outside of the membrane, then diffuse through the membrane and release the ion on the other side (Nobel, 1991). Another theory is that channels through the membrane exist. These channels have bindings sites where the ion moves through the membrane by moving from site to site within the channel. A third possibility is that the ion initially binds to a site on the outside of the membrane. The carrier molecule would then undergo a conformational change, moving

the ion to the inside of the cell. For each of these theories, energy must be used to move the ion (Nobel, 1991).

When carrier-mediated uptake is considered, the soil solution concentration of the ion is one of the most important controlling factors. As the soil solution concentration rises, the uptake rate eventually reaches a maximum. At this point, all the binding sites for an ion are filled and the uptake rate is at its maximum. This is similar to the principles of the Michaelis-Menten equation for enzyme kinetics. This equation is:

$$V = V_{max} * s/(K_m + s) \qquad (7)$$

where V is the velocity of the enzyme reaction, V_{max} is the maximum velocity of the reaction, s is the substrate concentration, and K_m represents the substrate concentration when $V = 0.5\ V_{max}$. Epstein and Hagen (1952) first used this equation to determine the potassium uptake kinetics of excised barley roots. Since then, Michaelis-Menten kinetics have been used to describe uptake kinetics for a wide variety of crops and ions. While Michaelis-Menten kinetics work well in the concentration range of nutrients found in the soil, they may not be applicable when wide ion concentration ranges are used. When wide concentration ranges have been used, uptake can appear to be multiphasic (Epstein, 1966; Raines and Epstein, 1967). Claassen and Barber (1974) rephrased the Michaelis-Menten equation to more accurately represent soil and plant parameters. The parameters V and V_{max} became I_n and I_{max} to represent the net ion influx rate and the maximum ion influx rate, respectively. The parameter s became C_l to represent the soil solution ion concentration. Nielsen and Barber (1978) also added a term, C_{min}, to represent the ion concentration in solution where net influx is equal to zero. Their equation became:

$$I_n=I_{max}*(C_l-C_{min})/(K_m+C_l-C_{min}) \qquad (8)$$

After the ions enter the cell, they move from cell to cell through bridges (plasmodesmata) until they reach the xylem. This pathway is called the symplastic pathway.

The second method of absorption is passive absorption which can be broken into two categories: 1. passive ion movement into the plant, independent of respiration energy, and 2. passive uptake along an energy-dependent electrochemical gradient. The first category involves the movement of ion into the plant through the free space (apoplasm) in the root cortex. This free space is divided into two sections, the "outer space" or voids and nonliving tissue in the cortex and the Donnan free space. The Donnan free space is the part of the total free space that is occupied by ions that are bound to the negative charges arising from the carboxyl groups in the root tissue (Briggs et al., 1958; Jansen et al., 1960). The outer free space (apoplasmic pathway) extends from the epidermis of the root to the endodermis. At the endodermis, the apoplasmic pathway encounters the Casparian strip, a layer of suberized material through which water and ions cannot move. At this point the ions must move into the symplasm and through the plasmodesmata into the steele of the root where they can enter the xylem.

The second category of passive movement is that passive uptake in response to an energy-dependent electrochemical gradient. When ions are taken up actively by the root a charge imbalance results between the free space outside of the cell and the cell cytoplasm. The ions in the free space outside the cell diffuse across the cell membrane in

an attempt to equalize this charge imbalance resulting in the movement of ions into the cell without the expenditure of metabolic energy.

The Barber - Cushman Nutrient Uptake Model

The Barber-Cushman nutrient uptake model (Barber and Cushman, 1981) is a mechanistic model that describes nutrient uptake by plants. A mechanistic model uses mathematical equations to describe both soil supply of nutrients and root growth in order to calculate nutrient uptake as opposed to regression models that use statistical methods to obtain coefficients for unknown processes occurring between the plant and its environment. The advantage of a mechanistic model is that individual parameters can be changed to simulate different situations, whereas a statistical model is only relevant for a certain set of conditions. A mechanistic model is more flexible and, therefore, more accurate in changing environments.

Development of the Model

In developing the model, the mechanisms of ion movement through the soil and ion uptake were considered. Both of these mechanisms have been discussed earlier. This section will discuss how these concepts work together in the model.

Radial diffusive flux and mass flow were described mathematically earlier. When supplying roots with nutrients these components work simultaneously and this can be described by the equation:

$$J_r = D_e \, \delta C_t / \delta r + v_o C_l \qquad (9)$$

where J_r is the ion flux to the root, D_e is the effective diffusion coefficient, C_t is the total labile concentration of the ion is the soil, r is the radial distance of diffusion, v_o is the rate of water flux to the root, and C_l is the concentration of the ion in the soil solution.

The equation:

$$\delta 2\pi\, rJ_r/\delta r = \delta 2\, \pi r\, \delta C_t/\delta t \qquad (10)$$

is used to account for conservation of solute and because the radial area decreases as r decreases. This can be simplified to:

$$\delta rJ_r/\delta r = \delta r\, \delta C_t\, /\delta t \qquad (11)$$

Substituting equation 9 into equation 11 gives:

$$\delta(rD_e\, \delta C_l/\delta r + r_o v_o C_l)/\delta r = r\, \delta C_l/\delta t \qquad (12)$$

To convert C_t to C_l, we use the equation $b\, \delta C_l = \delta C_t$. The resulting equation is:

$$1/r\ \delta/\delta r\ (rD_e\, \delta C_l/\delta r + r_o v_o C_l/b) = \delta C_l/\delta t \qquad (13)$$

The value r_o is the root radius. By rearranging this equation, it becomes:

$$\delta C_l/\delta t = 1/r\ \delta/\delta r\ (rD_e\, \delta C_l\, /\delta r + r_o v_o C_l/b) \qquad (14)$$

This is a continuity equation that will describe the concentration gradient that results from the root with time when used with the appropriate boundary conditions. The concentration at the root surface (C_{lo}) can also be calculated from this equation. In the calculation, the initial boundary condition is $C_l = C_{li}$, $r>0$, $t=0$.

In addition to the initial condition, inner and outer boundary conditions occur. The inner boundary condition, at the root surface where $r=r_0$, is found by assuming that ion uptake follows the Michaelis-Menten kinetics previously discussed. The inner

boundary condition states that ion influx is equal to the amount of the ion being supplied to the root by diffusive flux and mass flow. Thus, the inner condition is:

$$D_e b\, \delta C_l/\delta r + v_o C_l = I_{max}(C_l - C_{min})/(K_m + C_l - C_{min})$$

The outer boundary condition exists at the edge of the ion depletion zone in the soil. This condition is:

$$C_l = C_{li},\ r = r_1,\ t>0$$

if there is no competition for ions by roots. If competition for ions exists, then the boundary condition becomes:

$$J_r = 0,\ r = r_1,\ t>0$$

where r_1 is the mean half-distance between roots.

Because diffusion supplies part of the ions to the root, the concentration at r_o will decrease with time causing decreased influx with time. Hence, total uptake can be found by summing the influx with time using the equation:

$$T = 2\pi r_o L_o \int_0^{tm} J_r(r_o,S)ds \qquad (15)$$

where T is the total uptake, L_o is the initial root length, and $J_r(r_o,S)$ is the influx at the root surface, S. In order to account for new root growth, the equation must be modified to:

$$T = 2\,\delta r_o L_o \int_0^{tm} J_r(r_o,S)dS + 2\,\delta r_o \int_0^{tm} \delta f/\delta t \int_0^{tm-1} J_r(r_o,S)dSdt \qquad (16)$$

where $\delta f/\delta t$ is the root growth rate. This is the equation used to calculate uptake by roots growing in a uniform medium. It has to be solved numerically.

Assumptions

There are ten assumptions that are made when using the continuity equation.

1. The soil is homogenous and isotropic.
2. Moisture conditions are close to constant. In the ion flux calculations, it is assumed that there is no real moisture gradient perpendicular to the root. The moisture gradient is usually flat because water diffusion rate is generally high.
3. Roots only take up ions from the soil solution at the root surface.
4. Root exudates or microbial activity on the root surface do not affect uptake.
5. Mass flow and diffusion combine to move nutrients to the root surface.
6. Michaelis-Menten kinetics may be used to describe the relation between net influx and concentration.
7. Root are assumed to be smooth cylinders without root hairs or mycorrhizae.
8. D_e and b are assumed to be independent of concentration. (This is known to be invalid for some ions and for these, an average value for the range of interest can be used.)
9. Neither root nor plant age affect the influx characteristics.
10. Influx is independent of the rate of water absorption.

These assumptions are needed to simplify the calculations. While some are known to be invalid, there are ways to take variations into account.

Model Parameters

The Barber-Cushman model uses 11 parameters to determine ion uptake by plants. These parameters are:

1. D_e, the effective diffusion coefficient of the ion in soil, $cm^2\ s^{-1}$.
2. b, the ability of the diffusible ion concentration to buffer changes in the soil solution concentration, dimensionless.
3. C_{li}, the initial ion concentration in the soil solution, $\mu mole\ mL^{-1}$.
4. v_o, the water flux rate to the root, $cm\ s^{-1}$.
5. r_1, the mean half distance between root axes, cm.
6. r_o, the mean root radius, cm.
7. L_o, the initial root length, cm.
8. k, the root growth rate, $cm\ s^{-1}$.
9. I_{max}, the maximum influx rate of the ion at high concentrations, $\mu mole\ cm^{-2}\ s^{-1}$.
10. K_m, the solution concentration where influx = 0.5 I_{max}, $mmole\ L^{-1}$.
11. C_{min}, the solution concentration where there is no net influx, $mmole\ L^{-1}$.

These parameters can be separated into three groups: the soil supply parameters: D_e (1), b (2), and C_{li} (3); the root growth and morphology parameters: r_1 (5), r_0 (6), L_o (7), and k (8); and the uptake kinetic parameters: I_{max} (9), K_m (10), C_{min} (11), and V_o (4).

Verification of the Model

Chen and Barber (1990) tested the model at various pH levels by growing corn in a silt loam soil, measuring the amount of phosphorus taken up the plant, and comparing the observed phosphorus uptake to that calculated by the model. They found that the predicted phosphorus uptake agreed closely with observed phosphorus uptake when the form of phosphorus at each pH level was taken into account. This relationship was described by:

$$y = 0.93x + 0.44 \quad r^2 = 0.99 \qquad (17)$$

where x is the observed phosphorus uptake and y is the predicted phosphorus uptake. Blanco (1989) also provided verification of the model. He grew corn on seven soils including Andosols and Oxisols from Columbia and Mollisols from the U.S. He found close to a 1:1 agreement between observed and predicted phosphorus uptake. The equation used to describe this relationship was

$$y = 0.99x - 1.34 \quad r^2 = 0.995 \qquad (18)$$

where x is the observed phosphorus uptake and y is the predicted phosphorus uptake. These and other studies (Barber, 1984) provide proof that the model accurately describes phosphorus uptake by plants.

Because of the similarity between arsenic and phosphorus, the model should predict arsenic uptake by plants as well, provided that accurate measurements of the parameters can be made.

CHAPTER 1

THE EFFECT OF SOLUTION ARSENIC CONCENTRATION AND FORM ON THE GROWTH OF CANOLA

Introduction

Canola (*Brassica napus* L.) is an oilseed crop that produces a high quality, edible oil. The oil is low in saturated fat, thus it is becoming a popular cooking oil as consumer's health consciousness grows. Canola grows well on loam and sandy loam soils. In Louisiana, these soils are used for cotton production, hence, rotating cotton and canola may be attractive to producers wishing to optimize their land use. A possible limitation to canola production is the use of arsenic (As) compounds in cotton production. Bioavailable As from these agrichemicals may be present in elevated concentrations in the soil and thus affect canola growth.

Arsenic in virgin soils rarely exceeds 10 mg kg^{-1} (Shacklette and Boerngen, 1984, Selby et al., 1974, Vinogradov, 1959). However, As levels can reach high concentrations where there is a history of prolonged As use. Woolsen et al. (1971) compared As concentrations in 58 surface soils with histories of As application with nearby virgin soils. The experimenters found that the contaminated soils contained an average of 13-fold more As than the uncontaminated soils. In Louisiana, Ori et al. (1993) found that soils with histories of As application average about 23 mg As kg^{-1} as opposed to and average As content of 5-6 mg As kg^{-1} in virgin soils (Adriano, 1986.

Calcium arsenate was the major form of arsenic used in cotton production from the early 1900's to the mid 1960's. In the middle 1960's, organic arsenicals were

developed and replaced the inorganic forms. Organic forms of As used in cotton production include monosodium methanearsenate (MSMA) and disodium methanearsenate (DSMA). Monosodium methanearsenate has been reported to be more toxic to plants than DSMA (U.S.EPA, 1975). At common agricultural soil pH's, MSMA and DSMA both exist in the soil as the monovalent methanearsenate ion (MMA) and will degrade to arsenate [As (V)] with time (Hiltbold, 1975).

Arsenic's phytotoxicity is a result of its similarity to the phosphorus (P) ion. Arsenic can substitute for P in the plant but cannot mimic P's role in metabolism. A common As toxicity symptom is purpling in the shoot tissue identical to a P deficiency symptom. Various studies have shown that increasing As concentrations result in continued reduction in plant growth (Woolsen et al., 1971, Wallace et at., 1980).

While soil As concentration affects the As toxicity to plants, As speciation has also been shown to affect toxicity (Marin et al., 1991, Deuel and Swoboda, 1972, Reed and Sturgis, 1936). The most common inorganic forms of As in the soil are arsenate (As (V)) and arsenite (As (III)). In oxidized conditions, As(V) is the predominant ion while As(III) becomes more prevalent as reducing conditions occur. Arsenite has been found to be more soluble in soil and more toxic than arsenate. Masscheleyan et al. (1991) found a 25-fold increase in total solution As when soils were placed under reducing conditions. However, As (III) forms under reducing conditions that are not normally found in well-drained soils. Hence, As (III) should not be present in soil where canola is grown. Another aspect of arsenic speciation is its effect on As translocation in the plant. Several studies have shown As speciation affects As movement in plants. Marin et al.

(1992) showed that speciation affected As transport in rice. The experimenters found that dimethyl arsenic acid was translocated to the shoots of rice while MMA, As(V) and As(III) remained in the roots. Rumberg et al. (1960) found that while both DSMA and As (III) move in the plant, DSMA was more mobile. Sachs and Michael (1971) also found that arsenic compounds translocated differently within the plant. The effect of speciation on toxicity is believed to be responsible for differences in translocation. The more toxic As species slow plant metabolism faster than the less toxic species, thus reducing the amount of translocation of the more toxic As form.

Plants grown on As contaminated soil have shown varying tolerances to arsenic (Jacobs et al., 1970, Liebig, 1966). In general, crops similar to beans (*Phaseolus* spp.), most of the legumes, and rice (*Oryaz sativa* L.) are sensitive to As while plants such as carrots (*Daucus carota sativa*), tomatoes (*Lycopersicon esculentum*), wheat (*Triticum* spp.), and oats (*Avena sativa*) are tolerant (Adriano, 1986). Because canola is a relatively new crop, little research has been conducted to investigate the effects of As on its growth. However, a close relative of canola, wild mustard (*Brassica kaber*), has been shown to be sensitive to As (U.S.EPA, 1975).

This research should help define a potential limitation to canola growth in environments containing elevated levels of As. The objectives of this research were to determine I.) the effects of solution As speciation and As concentration on canola growth, ii.) the effects of speciation on As translocation in plants, and iii.). the effects of As speciation and concentration on nutrient uptake.

Materials and Methods

A 3 X 4 factorial study in a controlled climate chamber (25°C and 13 hours of 239 μmol s^{-1} m^{-2} light) was used. Three As forms (As (V), MSMA, and DSMA) and four concentrations (0, 0.02, 0.50, and 1.00 mg As L^{-1}) were used. While MSMA and DSMA exist in the soil as MMA, both forms were used in this study since both are commercially available and have been reported to affect plants differently. The As (V) form was supplied as sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$).

Canola was pregerminated for 7 days, then 4 seedlings per pot were transferred to 0.25 strength modified Hoagland's solution (Epstein, 1972). The pH of the system was adjusted to pH 6.5 with NaOH. The seedlings were allowed to acclimatize in the nutrient solution for another 7 days before the As treatments were added. Each As form-concentration combination was added to pots containing 1.0 L of nutrient solution. Nutrient solutions with the specific As form-concentration combinations were changed every 2 days to avoid changes in As speciation. Pots were stirred by bubbling with air. Three replications were used. Plants were grown for 12 days in the As form-concentration treatments then harvested. Root length was measured using a line intersect method (Tennent, 1975). Shoots and roots were dried for 24 hr at 65°C, weighed, then ground with a Wiley mill to pass a 20 mesh screen. After grinding, plant tissues were digested with concentrated H_2SO_4 and 30% H_2O_2 (Adler and Wilcox, 1985). Digests were diluted with deionized, distilled water and analyzed for As by atomic absorption spectrophotometry with hydride generation (Masscheleyn et al., 1991). Acid blanks were used to determine the As content of the H_2SO_4. The

concentration of the As in the diluted acid blanks was below the detection limit (2.0 μg L^{-1}). Plant nutrient concentrations in the digests were determined by inductively coupled argon plasma spectroscopy.

Results and Discussion

Arsenic Compounds Effects on Plant Growth

Effect of As Form and Concentration on Root Length and Root Dry Weight.

Root length was affected by solution As form and concentration (Figure 1.1). Root lengths in all treatments were significantly shorter than in the control. Root length decreased as solution As concentration in each As form increased to 0.50 mg L^{-1}. At the 1.00 mg L^{-1} concentration, root length increased. This increase was greatest for the inorganic As (76.5 cm), while the root length increases due to MSMA and DSMA were similar (MSMA, 41.3 cm; DSMA 32.4 cm). The different forms of As showed no significant difference in root length at the 0.02 mg L^{-1} concentration, indicating that at this level of As, both inorganic and organic forms were equally efficient at slowing root growth. However, at 0.50 and 1.00 mg L^{-1} of As, root length due to MSMA and DSMA were significantly less than that with inorganic As. This would imply that the organic forms of As were more efficient in slowing the root growth. As expected, no significant difference in root length occurred between the MSMA and DSMA treatments.

Root dry weight followed the same trends as root length (Figure 1.2). All treatments were significantly less than the control. However, there were no significant differences in the root dry weights within As form or concentration. Any differences in trends between root dry weight and root length would signal a change in root

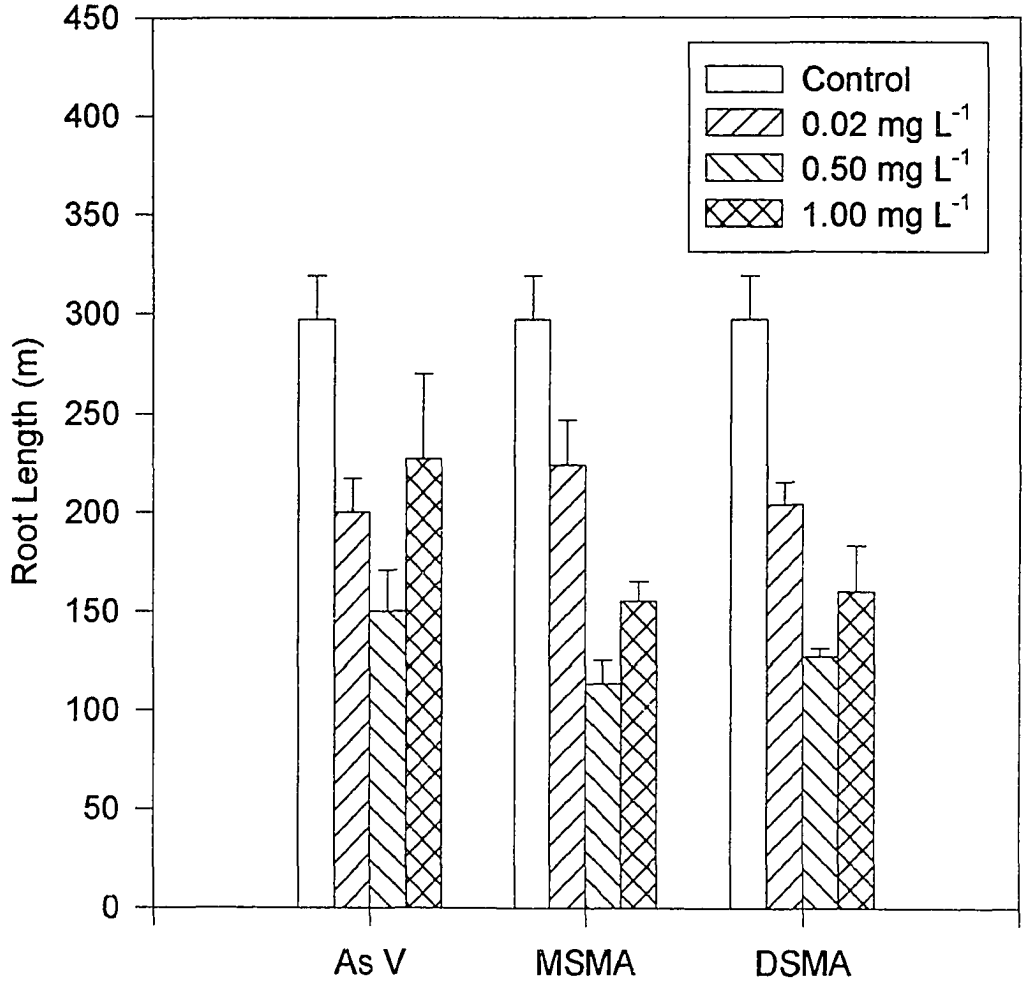

Figure 1.1 Effect of As concentration and form on the root length of 28-day-old canola.

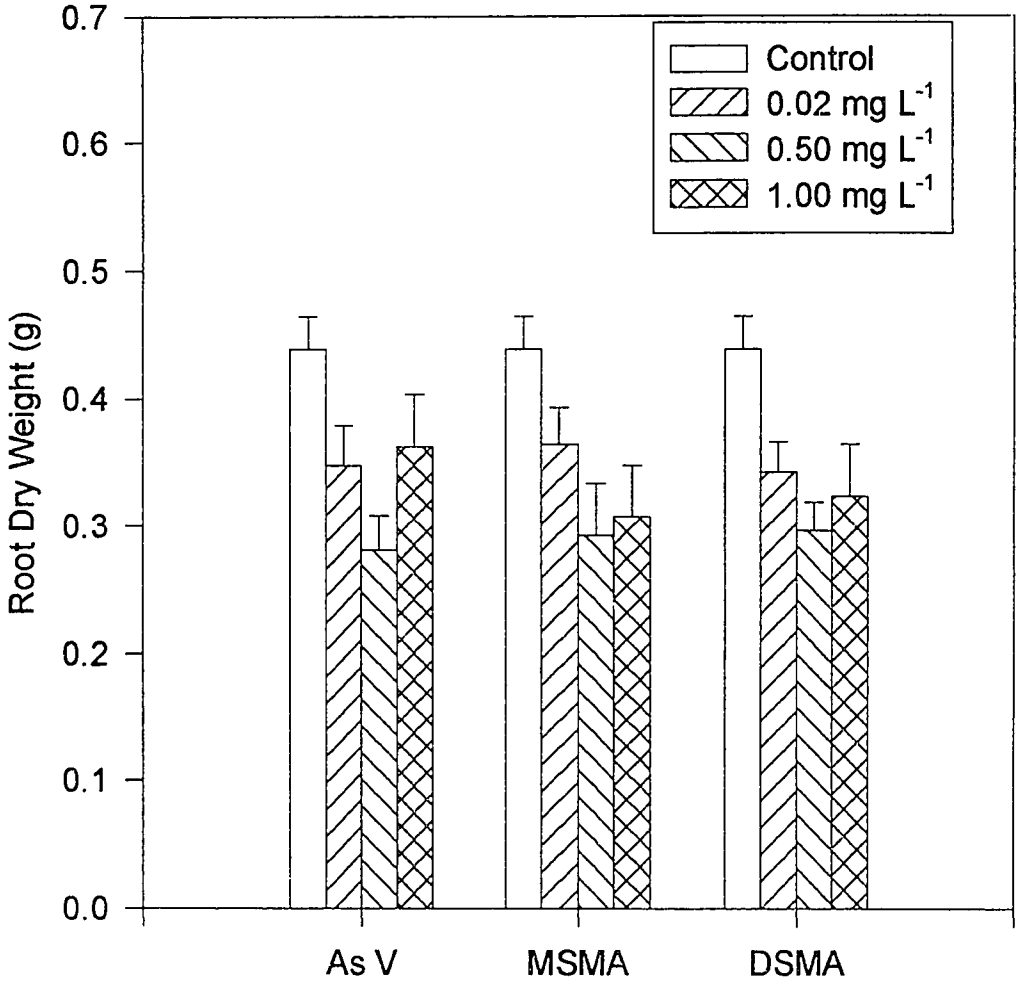

Figure 1.2 Effect of As concentration and form on the root dry weight of 28-day-old canola.

morphology caused by the As form. Since trends between root length and root dry weight were similar, As form did not appear to affect root morphology.

It can be speculated that the increase in root dry weight and root length at 1.00 mg L^{-1} of As may be a plant response to decreased ion uptake. As root growth decreases, the plant nutrient demand may exceed nutrient uptake. The plant responds to this difference by increasing root growth to forage for nutrients. In each As form, the root dry weight:shoot dry weight ratios at 1.00 mg L^{-1} were higher than the ratios in the 0.02 and 0.50 mg L^{-1} of As. It is interesting to note that as the plant increases root growth to increase nutrient uptake, it could also increase As uptake, thus, increasing the stress conditions.

Effect of As Concentration and Form on Shoot Dry Weight

Arsenic form and concentration affected shoot dry weight differently (Figure 1.3). The shoot dry weights due to inorganic As at any concentration were not significantly different from those of the control. The shoot dry weights were not significantly affected by MSMA or DSMA at 0.02 mg L^{-1} As but were significantly reduced at 0.50 and 1.00 mg As L^{-1}. This difference between organic and inorganic As forms may be a result of the differences in the translocation of the As forms. The [shoot As]:[root As] (S/R) ratios (Table 1.1) show the organic As forms to be more readily translocated than the inorganic form. Hence, the higher concentration of As in the shoot tissue receiving organic As may reduce shoot growth. Purpling of the lower leaves and stem, an As toxicity symptom, due to organic As treatments was also noted.

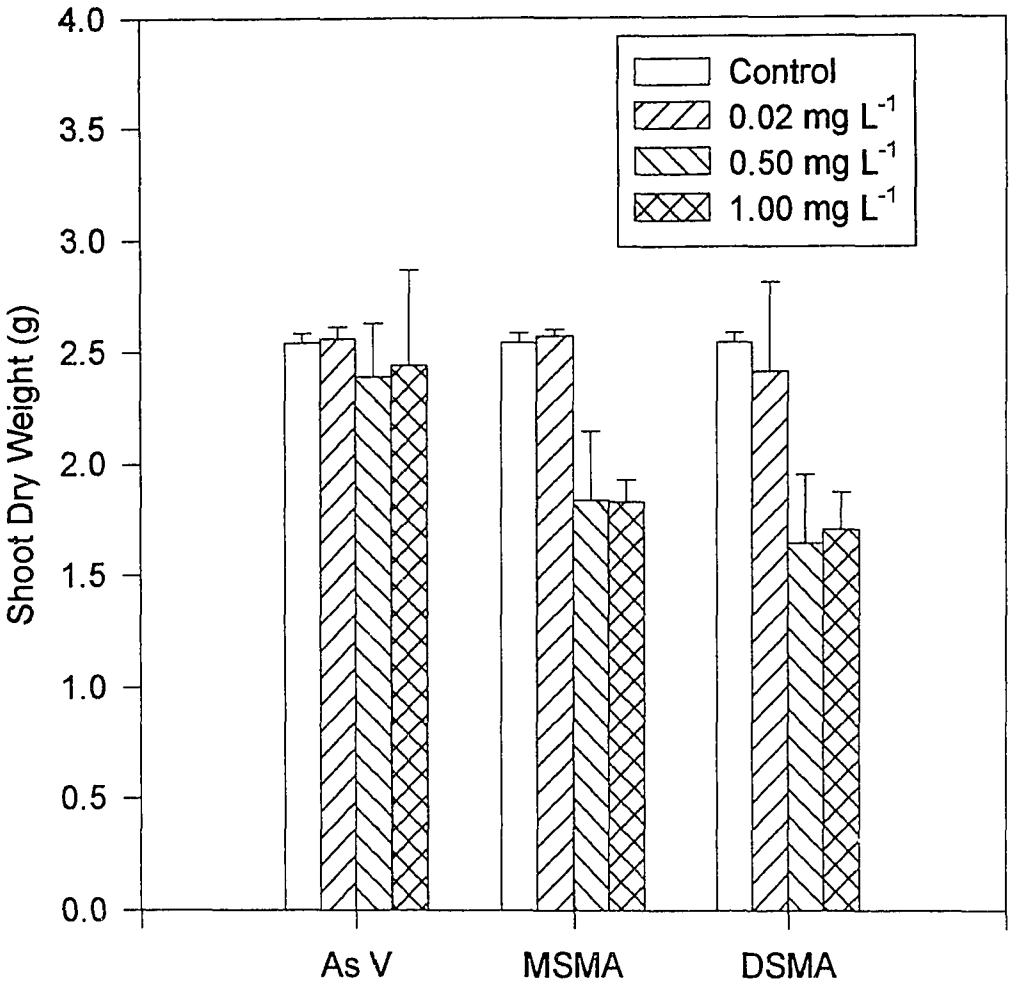

Figure 1.3 Effect of As concentration and form on shoot dry weight of 28-day-old canola.

Table 1.1 Root and shoot arsenic concentrations and the shoot arsenic:root arsenic ratio (S/R) for the As V, MSMA, and DSMA treatments.

As added		Root As Conc..	Root As Uptake	Shoot As Conc..	Shoot As Uptake	S/R
Form	Conc. mg L^{-1}	µg g^{-1}	µg	µg g^{-1}	µg	
	0.00	BDL*	BDL	BDL	BDL	
As V	0.02	5.80a†	2.03a	2.28a	5.84a	0.39
	0.50	8.00a	2.24a	2.41a	5.76a	0.30
	1.00	7.20a	2.59a	2.19a	5.34a	0.30
MSMA	0.02	2.50a	0.90a	1.83a	4.70a	0.73
	0.50	5.47ab	1.59a	1.95a	4.12a	0.41
	1.00	10.30b	3.19a	5.29b	9.68b	0.51
DSMA	0.02	3.26a	1.11a	2.64a	6.36a	0.81
	0.50	5.31b	1.59a	3.69a	6.05a	0.70
	1.00	6.83b	2.19a	4.99b	8.48a	0.73

*BDL represents a value below the detection limit

† Values within As form followed by the same letter are not significantly different (α=0.05)

Shoot dry weights due to organic As levels of 1.00 mg L^{-1} were approximately equal to those at 0.50 mg L^{-1}, while As concentration in the shoot tissue increased (Figure 1.3 and Table 1.1). This would imply that solution As concentrations greater than 0.50 mg L^{-1} did not have any additional effect in slowing shoot growth. As seen in the root length measurements, no significant differences in shoot dry weight were found between MSMA and DSMA treatments.

Effect of As Form and Concentration on As Accumulation and Translocation

Accumulation of As in root and shoot tissue depended on As form and concentration (Table 1.1). Shoot and root As concentrations due to inorganic As(V) did not increase with increasing solution As concentration. Shoot As concentration due to 1.00 mg L^{-1} As(V) was less than that due to 0.50 mg L^{-1} As(V) (Table 1.1). Arsenic concentrations in the roots also followed this trend. A possible explanation for this decrease in root As lies in the As uptake mechanism. Oxidative phosphorylation provides the energy for active As uptake. Arsenic uncouples this oxidative phosphorylation (Amburgey, 1967) thus, as total As in the roots, where active uptake occurs, increases, the amount of energy available for As uptake decreases. Hence, a dilution effect occurs as the root growth continues with a decreased rate of As uptake. This concept is supported by the total root As levels increasing with solution As concentration (Table 1.1).

Unlike tissue As concentrations due to As(V) treatments, shoot As concentrations due to MSMA ($y=0.76+4.74x$, $r^2=0.72$, $P\leq0.05$) and DSMA ($y=1.51+4.37x$, $r^2=0.40$, $P\leq0.05$) tended to increase linearly as solution As concentration

increased. Root As concentrations also followed this trend (MSMA: y=0.75+11.26x, r^2=0.69, P≤0.05; DSMA: y=0.80+7.97x, r^2=0.77, P≤0.05). These linear increases can be also be related to the uptake mechanisms. Two uptake mechanisms can affect the organic forms of As. Active uptake, as with the inorganic form, and passive uptake, across the cell membrane, can occur with organic As. The methyl group present on the organic arsenicals eases the diffusion of these ions across the root cell membrane (Ross and Lembi, 1985). Hence, while the active uptake is slowed or stopped, the organic forms can still move into the plant by diffusion and tissue concentrations will increase as seen due to the organic arsenic.

Arsenic was found in greater concentrations in the roots than in the shoots of all plants (Table 1.1). This implies that As is not very mobile in the plant. The amount of translocation can be determined by comparing the shoot As:root As (S/R) resulting from each treatment (Table 1.1). The organic As forms were more mobile in canola than the inorganic form (Table 1.1). Within treatment concentrations, the shoot:root ratios due to organic forms were higher than those due to As (V). The shoot:root ratios due to each As form decreased at concentrations above 0.02 mg As L^{-1}. These decreases in the shoot:root ratio are probably an effect of the toxicity of the As form. Hence, as plant As concentrations increased, the plant metabolism was apparently reduced and translocation was impeded.

Effect of As Form and Concentration on Nutrient Uptake

Since As affected shoot and root growth, nutrient uptake might also have been affected. It can be speculated that as nutrient demand by the plant exceeds nutrient

uptake, the plant could attempt to overcome this deficiency by increasing nutrient uptake. Hence, plant nutrient concentrations may increase as As increases. However, the opposite could also occur if As was slowing active transport of nutrients into the plant or if the As was competing for uptake especially with P. Shoot levels of Ca, K, P, Mg, B, Mn, Zn, Cu, and Fe (Appendix) were compared with nutrient concentrations due to 0.00 mg As L^{-1}. Of these nutrients, Ca, P, and Zn showed definite trends due to the organic As treatments. Regression analysis suggested a linear increase in shoot Ca concentration due to both MSMA ($y=28374+7881x$, $r^2=0.55$, $P\leq0.05$) and DSMA ($y=28058+6371x$, $r^2=0.47$, $P\leq0.05$) treatments (Figure 1.4). The argument can be made that these increases are more likely an concentration effect due to the reduced shoot growth of the canola, however, if this was the case, increases in the shoot content of all of the nutrients examined should have increased.

Shoot P concentrations also increased in the organic treatments (Figure 1.5). In the MSMA ($y=8178+4293x$, $r^2=0.54$, $P\leq=0.05$) and DSMA ($y=7678+5394x$, $r^2=0.55$, $P\leq0.05$) treatments, shoot P concentrations tended to increase as solution As concentration increased. Since As can substituting for P in the plant but is unable to carry out P's role in energy transfer, the plant reacted as if there is a P deficiency. Thus, as plant As increases, the plant reacts by increasing P uptake. It is interesting to note that by increasing P uptake, the plant may also increase As uptake due to the similarity of the ions. The same arguments made for the increases in shoot Ca also apply for shoot P.

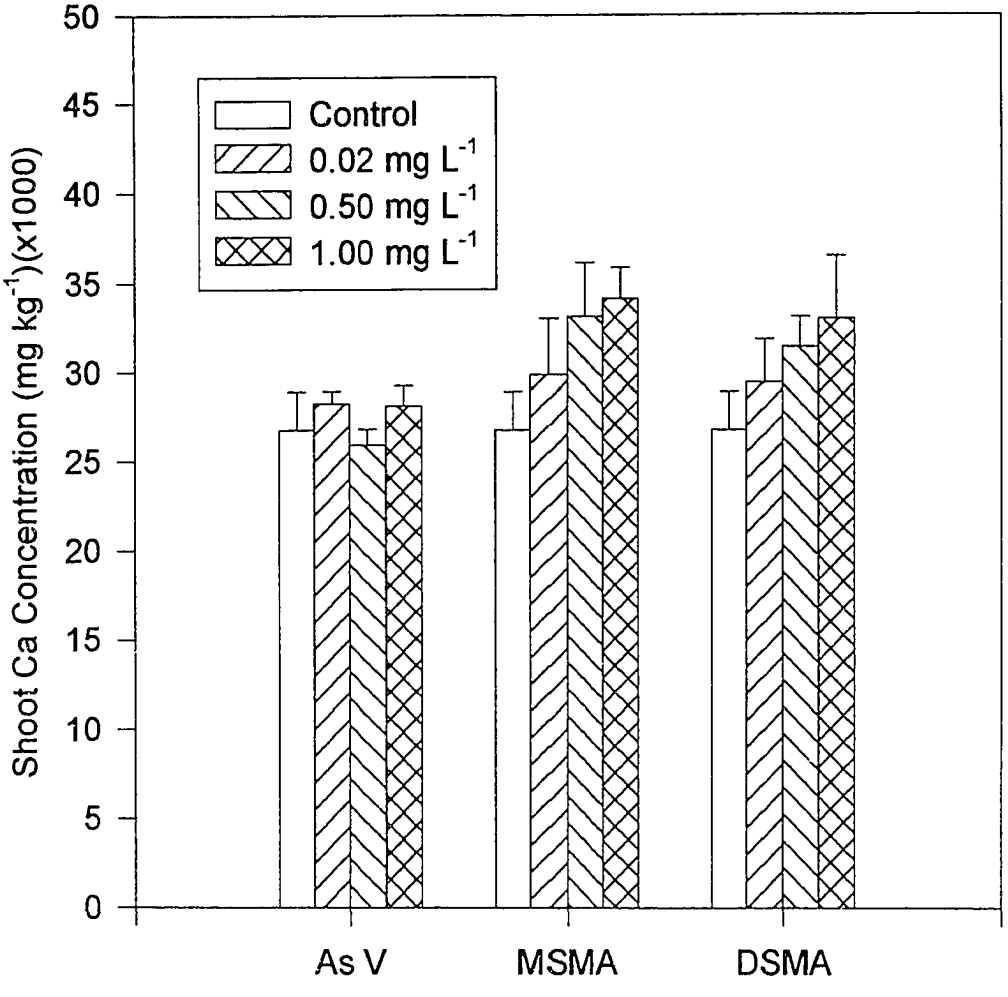

Figure 1.4 Effect of As concentration and form on shoot Ca concentrations in 28-day-old canola.

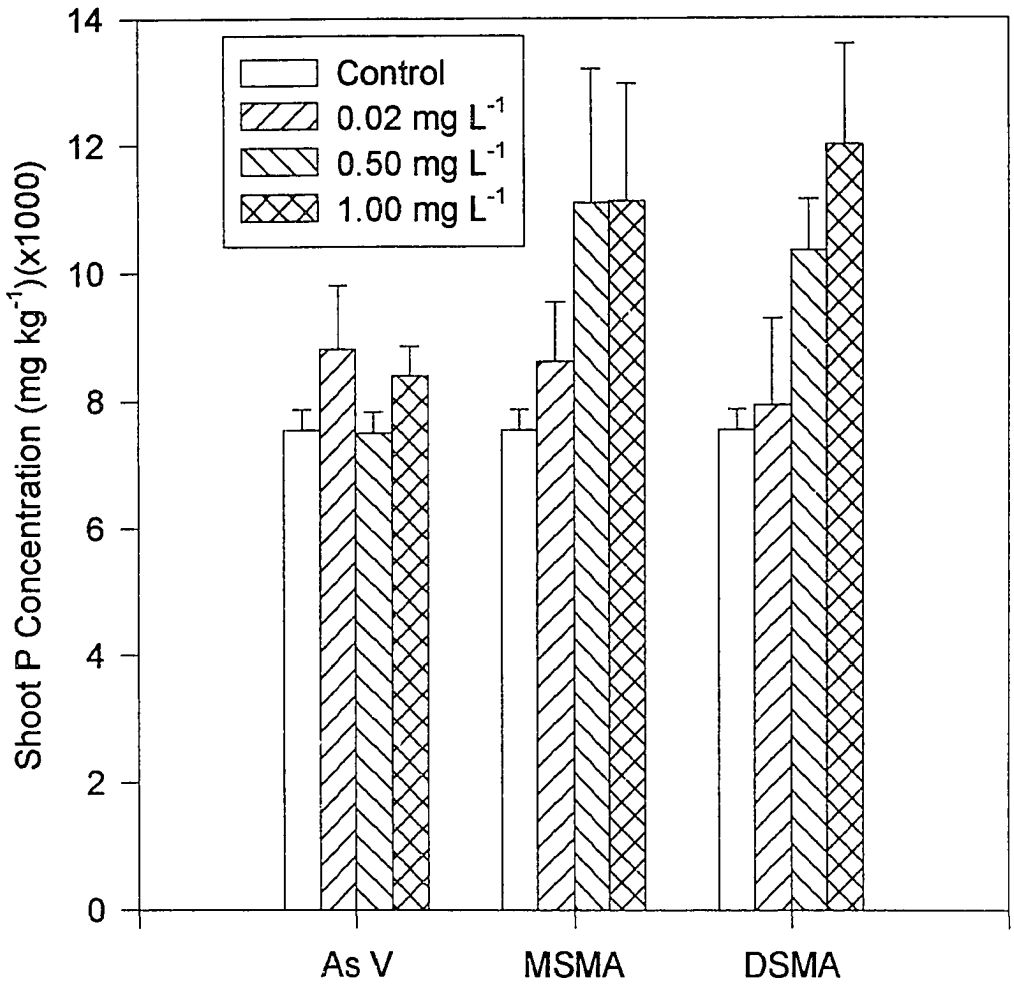

Figure 1.5 Effect of As concentration and form on shoot P concentrations in 28-day-old canola.

Contrary to Ca and P, shoot Zn concentrations tended to decrease due to organic As as solution As increased (MSMA: $y=30.66-16.24x$, $r^2=0.46$, $P\leq0.05$. DSMA: $y=34.35-22.30x$, $r^2=0.69$, $P\leq0.05$) (Figure 1.6). Two possible explanations are suggested for this decrease. The first involves ion competition for uptake. Zinc is known to compete with P for uptake (Olsen, 1972), thus the decrease in Zn could be due to the increase in shoot P concentrations. Zinc could also be competing directly with As for uptake since the P and As ions are similar. A second possible reason for the decrease in Zn uptake could be that As slowed the active transport of Zn into the plant, thus leading to decreasing Zn tissue concentrations.

Conclusions

Canola oil is becoming popular with consumers because of its high quality, low saturated fat properties. With this increase in popularity comes an increase in demand providing needed diversification for farmer operations.

In this study, we have attempted to define a possible limitation to growing canola as an alternative crop. We have shown that both the concentration and form of As in solution have significant effects on canola growth. Inorganic As(V) in this hydroponic study affected root growth while organic MSMA and DSMA affected both shoot and root growth. We also showed that inorganic As (V) did not seem to affect ion uptake while the organic MSMA and DSMA appeared to stimulate Ca and P uptake and depress Zn uptake.

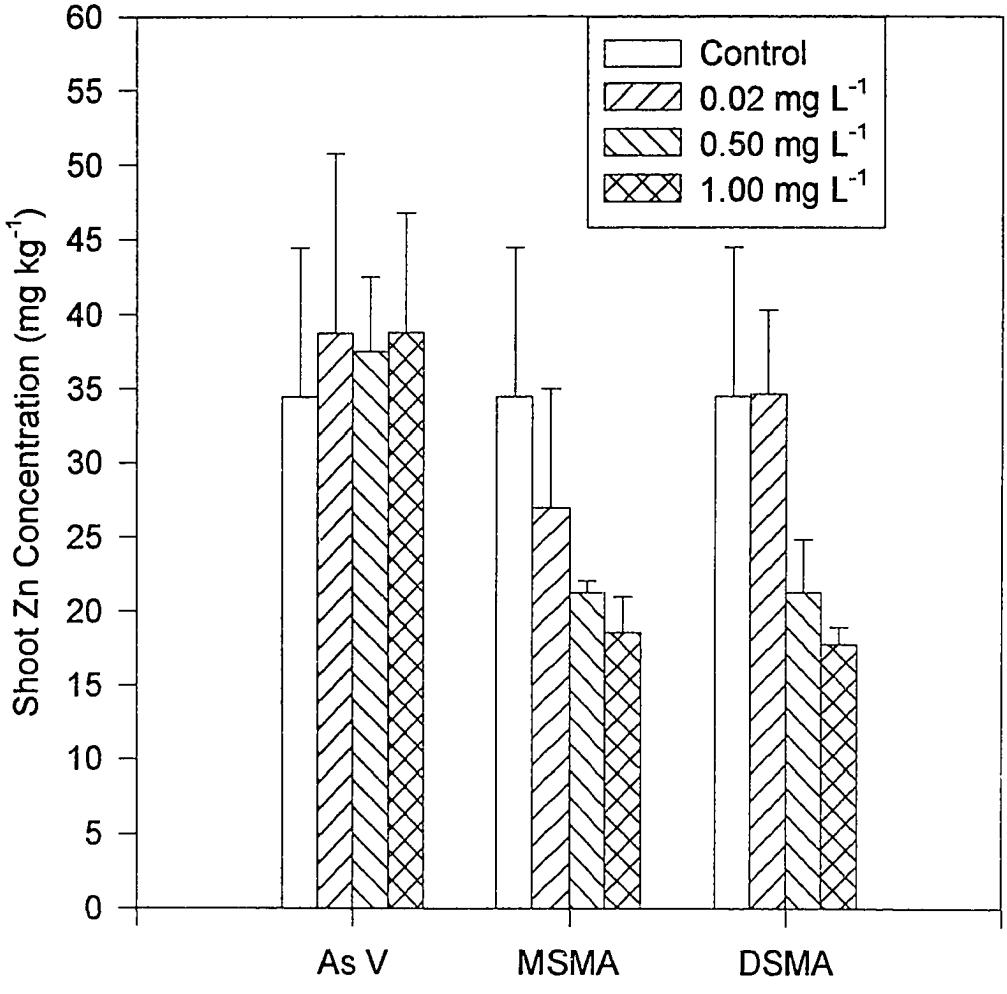

Figure 1.6 Effect of As concentration and form on shoot Zn concentrations in 28-day-old canola.

CHAPTER 2

ARSENIC SUPPLY CHARACTERISTICS IN FOUR COTTON-PRODUCING SOILS

Introduction

Arsenic is a naturally occurring element and can be found in all soils. Total As concentrations in virgin soils average 5 mg kg^{-1} and rarely exceed 10 mg kg^{-1} (Adriano, 1986; Ori et al., 1993; Walsh and Keeney, 1975; Woolsen et al., 1971). In agricultural soils, however, the As level may be much higher. Agricultural soils in Louisiana average 23 mg kg^{-1} As due mainly to the use of As-containing pesticides and defoliants in cotton production (Ori et al., 1993). Although several plant species of agronomic importance are known to be sensitive to As (Adriano, 1986), little research has addressed As bioavailability in cotton-producing soils. This could become an important management factor, however, if cotton is rotated with other crops.

Soil levels of As have traditionally been studied on a total basis with regard to contamination or on an extractable basis with regard to plant availability. However, these approaches may not be adequate to characterize the dynamic nature of soil As as it relates to uptake by plant roots. Recently, mechanistic models have been developed that accurately predict nutrient uptake by plant roots growing in soil (Barber, 1984). To predict uptake, these models mathematically describe the soil supply characteristics of the nutrient, root growth, changes in morphological characteristics of the roots, and uptake kinetics of the plant for the nutrient (Barber, 1984). While these models have been used extensively to predict the uptake of many plant nutrients (Barber, 1984), little

information is available on modeling the uptake of other ions in the soil. As shown in several studies (Barber, 1984), the soil supply characteristics exert a large influence on uptake. Therefore, the first step in modeling As uptake by plants is to characterize the relationships among the different As phases in the soil.

When As is added to soil, it may remain in soil solution, be adsorbed on the solid phase, be specifically adsorbed, and precipitate. Soil As is commonly present as As^{5+} under oxidized conditions and As^{3+} under reduced conditions. Since the reduction of As^{5+} to As^{3+} is slow, As^{3+} generally will not be present in well-drained soils where cotton is grown (Masscheleyn et al., 1991). Under highly reduced conditions, arsine may be present, but this form is not common in agricultural soils. Arsenic preferentially forms surface complexes or precipitates with Fe, Al, Mn, and Ca (Atkins and Lewis, 1976; Jacobs et al., 1970; Woolsen et al., 1971, Hess and Blanchar, 1976).

The As in soil solution is able to move through the bulk soil to plant roots by mass flow and diffusion (Barber, 1962). Movement by mass flow occurs when solution As moves in the convective flow of soil water. Diffusion of As is due to the random kinetic movement of the ion (Brownian movement) in response to a concentration gradient that can be created by root absorption. Solution As at the root surface is initially in equilibrium with diffusible As in the bulk soil. The total diffusible As is that fraction of the As that is considered plant available and includes both solid-phase As and solution phase As (Van Rees et al., 1990). Hence, the change in these phases with As addition is of interest.

Due to the complexity of As reactions in soil, adequate characterization of the soil As supply is difficult. One approach is to mathematically describe the changes of both soil solution and solid-phase As after As addition. Working with P, an element with similar chemical behavior in the soil, Kovar and Barber (1988) used this approach to investigate the relation of both soil solution P and resin-exchangeable P with P addition in 33 diverse agricultural soils. They found that resin-exchangeable P, considered a measure of total diffusible P, increased linearly with P addition and that solution P increased curvilinearly with P addition. Currently, little research has been done to assess the effect of As addition on the different As phases in the soil. Based on the results of Kovar and Barber (1988) and the similarity of P and As chemical behavior in soil, a comparable approach for characterizing As supply in soil would provide useful information. Therefore, the objectives of this study were to: I.) determine the relationships among solution phase As, resin-exchangeable solid-phase As, and As addition, and ii.) examine soil properties that influence these relations.

Materials and Methods

Four diverse soils commonly used for cotton production in Louisiana were collected from the upper 15 cm of the profile, sieved to 2 mm particle size, and analyzed. The soils were Commerce silt loam (fine-silty, mixed, nonacid, thermic Aeric Fluvaquent), Gigger silt loam (fine-silty, mixed, thermic Typic Fragiudalf), Rilla silt loam (fine-silty, mixed, thermic Typic Hapludalf), and Sterlington silt loam (coarse-silty, mixed, thermic Typic Hapludalf). Initial soil analyses included particle size analysis (Day, 1982), organic matter content by acid-dichromate oxidation (Nelson and

Sommers, 1982), NH_4Ac-exchangeable cations, DTPA-extractable Fe and Mn, free iron oxides (Mehra and Jackson, 1960), exchangeable Al (Barnhisel and Bertsch, 1982), and pH in water. Water content at -33 kPa tension "field capacity" was determined by the pressure plate method (Klute, 1986). Total As was determined by a HNO_3-H_2SO_4 method (Ganje and Rains, 1982). Values for the initial chemical and physical properties can be found in Table 2.1.

Five rates of As (0, 50, 100, 150, and 200 mg kg^{-1}) were applied to the soils as sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$). The As salt was dissolved in deionized water, then applied and thoroughly mixed with the soil. The soils were allowed to equilibrate for 30 d. During the equilibration period, water content was maintained at 80% of the water held at -33 kPa tension. Three replications were used.

A column displacement method was used to determine solution As. This method accurately describes the unaltered composition of the soil solution (Adams, 1974). A 500-g sample (oven-dry weight) of the equilibrated soil was packed into a plexiglass column to a density of approximately 1.3 Mg m^{-3}. Filter paper was placed on the top of each soil column. Deionized water was added to each column at a rate of 4 mL h^{-1} until the soils reached "field capacity" (-33 kPa tension) water content. The samples were allowed to equilibrate for 24 h, then 40 mL of deionized water were added at a rate of 4 mL h^{-1}. The displaced solution was collected and filtered through a 0.20 μm filter. The solutions were analyzed by inductively coupled plasma spectroscopy (ICP). If As concentration was near the ICP detection limit (0.03 mg kg^{-1}), atomic absorption

Table 2.1 Initial soil chemical and physical properties of four cotton-producing soils used to determine the As soil supply characteristics

Soil Property	Soil Type			
	Commerce silt loam	Gigger silt loam	Rilla silt loam	Sterlington silt loam
Water Content, %†	19.2	23.6	18.8	16.1
pH	5.2	4.4	5.6	6.4
Organic Matter, %	0.30	0.23	0.28	0.15
Clay Content, %	13	10	5	4
Total As, mg kg^{-1}	4.2	11.2	5.2	9.8
Bray P2, mg kg^{-1}	240	57	74	109
Exch K, mg kg^{-1}	214	104	175	219
Exch Ca, mg kg^{-1}	1296	864	678	367
Exch Mg, mg kg^{-1}	239	179	89	35
DTPA-Ext. Fe, mg kg^{-1}	95.4	31.6	31.7	22.4
DTPA-Ext. Mn, mg kg^{-1}	3.8	43.5	8.7	1.2
Exch Al, mg kg^{-1}	7.27	15.73	10.31	5.06
Fe_2O_3, mg kg^{-1}	3355	6376	5871	2684

† determined at -33 kPa tension

spectroscopy with hydride generation (Ganje and Rains, 1982) was used. The change in soil solution As with As addition was characterized using nonlinear regression (SAS Inst., 1990).

Anion-exchange resin was used to determine total diffusible As. The resins are thought to act as a sink for As much the same as a plant root. A modified method of Amer et al. (1955) was used. A 0.5-g sample (oven-dry weight basis) of the moist, equilibrated soil, 5.0 g of Dowex 1x8 Cl^- saturated exchange resin (dia. >0.425 mm), and 100 mL of deionized water were added to a 400-mL plastic bottle. The samples were shaken for 24 h to desorb As from the soil. The soil and resin were separated by washing the soil from the resin. The resin was then shaken with 50 mL of 1 M HCl for 6 h to desorb As from the resin. The solutions were filtered through a 0.45 μm filter. As before, As in the solution was determined by ICP. If As concentration was near the ICP detection limit (0.03 mg kg^{-1}), atomic absorption spectroscopy with hydride generation was used (Ganje and Rains, 1982).

Since the exchange resin removes both ions in soil solution and ions adsorbed on the solid phase, resin-exchangeable solid-phase As values were calculated by subtracting solution As concentrations from the total diffusible As levels. The change in resin-exchangeable solid-phase As with As addition was characterized using nonlinear regression (SAS Inst., 1990).

Results and Discussion

Total initial As concentrations in the Rilla (5.17 mg kg^{-1}) and Commerce (4.15 mg kg^{-1}) soils were not higher than those normally found in virgin soils (5 mg kg^{-1},

Adriano, 1986). However, the total As concentrations in the Sterlington (9.75 mg kg^{-1}) and the Gigger (11.22 mg kg^{-1}) soils were twice the expected levels in untreated soils. These elevated levels of total As were not reflected by higher solution As in the untreated soils. Despite having similar total As concentrations, solution As in the Sterlington soil ($7.4x10^{-3}$ g m^{-3}) was more than 2-fold that in the Gigger soil ($2.7x10^{-3}$ g m^{-3}).

Effect of As Addition on Solution As Levels

Soil solution concentrations increased curvilinearly with As addition to the Commerce, Rilla, and Sterlington soils (Figure 2.1). Similar to the relation of solution P to added P (Kovar and Barber, 1988), the change in solution As levels with As addition was described by the equation $As_{sol}=ax^c+d$, where As_{sol} is the As concentration in soil solution, x is the amount of As added, and a, c, and d are regression coefficients. The value of "a" (which ranged from $2.25x10^{-7}$ to $1.26x10^{-3}$) describes the linearity of the increase in solution As, "c" (which ranged from 1.1 to 3.19) describes the curvilinearity of the relation ("c" values increasing from 1.0 indicate greater curvilinearity), and "d" (which ranged from $2.63x10^{-3}$ to $7.40x10^{-3}$ g m^{-3}) is the initial As concentration in solution. Values of the regression coefficients for each soil are shown in Table 2.2. The curvilinearity of these relationships indicates that relatively more As remained in solution as As addition increased and suggests greater potential availability to plant roots.

In contrast, solution As in the Gigger soil increased negligibly with the addition of As (Figure 2.1). The Gigger soil had a pH of 4.41 and high levels of

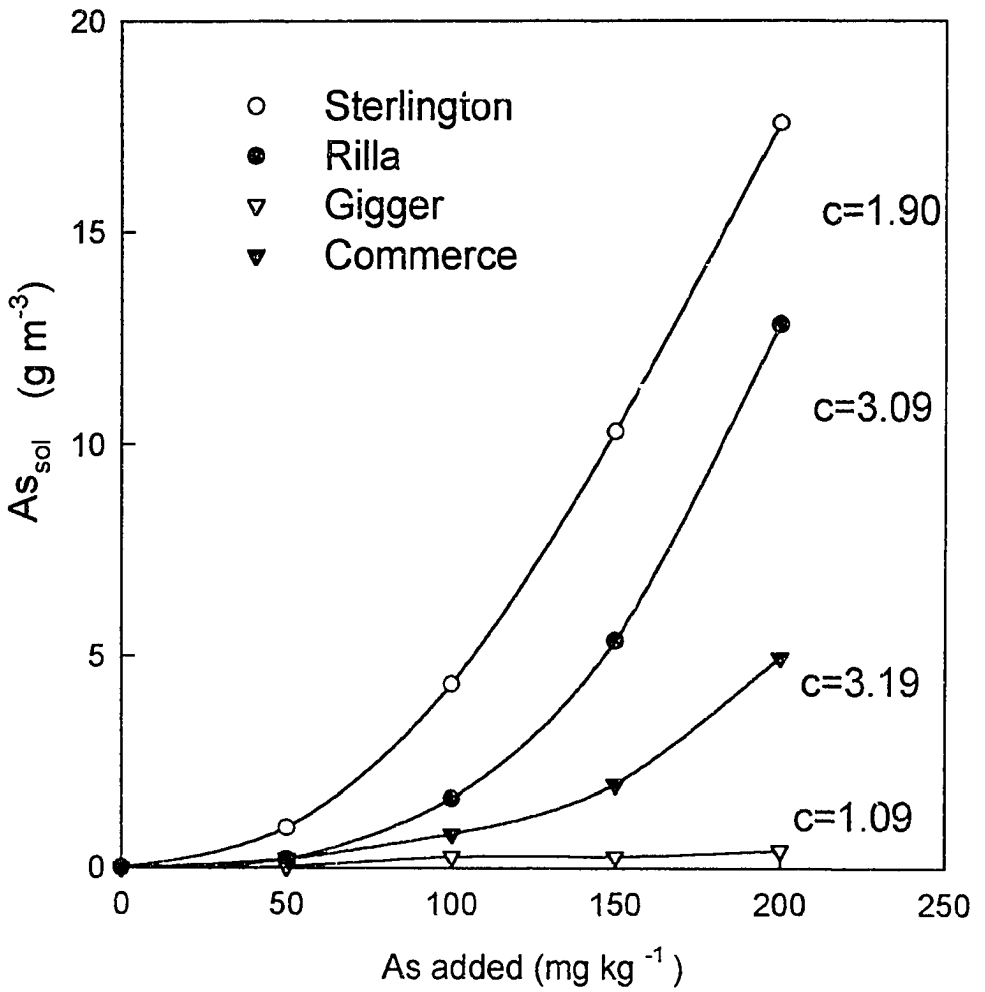

Figure 2.1 Relation between As added and As in soil solution of four soils. Observed values fit the equation: $As_{sol}=a(As\ added)^c+d$.

Table 2.2 Equations for the relations between solution As (As_{sol}), resin-exchangeable solid phase As (As_{resp}), total diffusible As (As_{td}), and added As for each soil.

Commerce

$$As_{resp} = 0.29\ (\text{As added})^{0.86} + 1.23$$

$$As_{sol} = 2.25E\text{-}7\ (\text{As added})^{3.19} + 2.75E\text{-}3$$

$$As_{td} = 19.28\ (As_{sol})^{0.39}$$

Gigger

$$As_{resp} = 0.14\ (\text{As added})^{0.99} + 0.61$$

$$As_{sol} = 1.26E\text{-}3\ (\text{As added})^{1.09} + 2.73E\text{-}3$$

$$As_{td} = 55.11\ (As_{sol})^{0.88}$$

Rilla

$$As_{resp} = 3.02\ (\text{As added})^{0.34} + 0.376$$

$$As_{sol} = 1.54E\text{-}6\ (\text{As added})^{3.09} + 2.60E\text{-}3$$

$$As_{td} = 15.08\ (As_{sol})^{0.34}$$

Sterlington

$$As_{resp} = 13.42\ (\text{As added})^{0.048} + 1.17$$

$$As_{sol} = 7.50E\text{-}4\ (\text{As added})^{1.90} + 7.40E\text{-}3$$

$$As_{td} = 15.98\ (As_{sol})^{0.28}$$

DTPA-extractable Mn. These conditions could lead to formation of Mn-As complexes (Hess and Blanchar, 1976). This is supported by a decrease of DTPA-extractable Mn from 58 mg kg^{-1} to 32 mg kg^{-1} as the amount of added As increased from 0 mg kg^{-1} to 200 mg kg^{-1} (Figure 2.2). When Mn-As complexes form, As is removed from solution, resulting in little increase in solution As with As addition.

Since the soils differed in the degree to which added As remained in solution, regression analysis was used to evaluate the effect of soil chemical properties on changes in solution As concentration. It would be advantageous if the potential As availability in a soil could be predicted by an easily-measured soil property. The regression coefficient "c" was compared with organic matter content, clay content, pH, exchangeable cations, initial solution As, resin-exchangeable solid-phase As, DTPA-extractable Fe and Mn, free iron oxides, and exchangeable Al. DTPA-extractable Mn and initial solution As concentration were correlated with the "c" value of the soils. For these four soils, the relationship was described by the equation $c=4.3-5.6x10^{-2}Mn-315.8As_{sol}$ ($r^2=0.99$, significant at the 0.05 level).

Initial solution As concentrations (As_{sol}) were inversely related to the "c" values of the soils. High initial levels of solution As suggest a lack of adsorption sites with an affinity for As, thus a small "c" value results, as seen in the Sterlington soil. While a significant relationship exists between the initial solution As and the "c" value, this relationship is likely a reflection of the adsorption properties in the soil, rather than the initial solution As concentration. A larger number of soils would be needed to confirm the relation of initial solution concentration and "c" values. No significant relationship

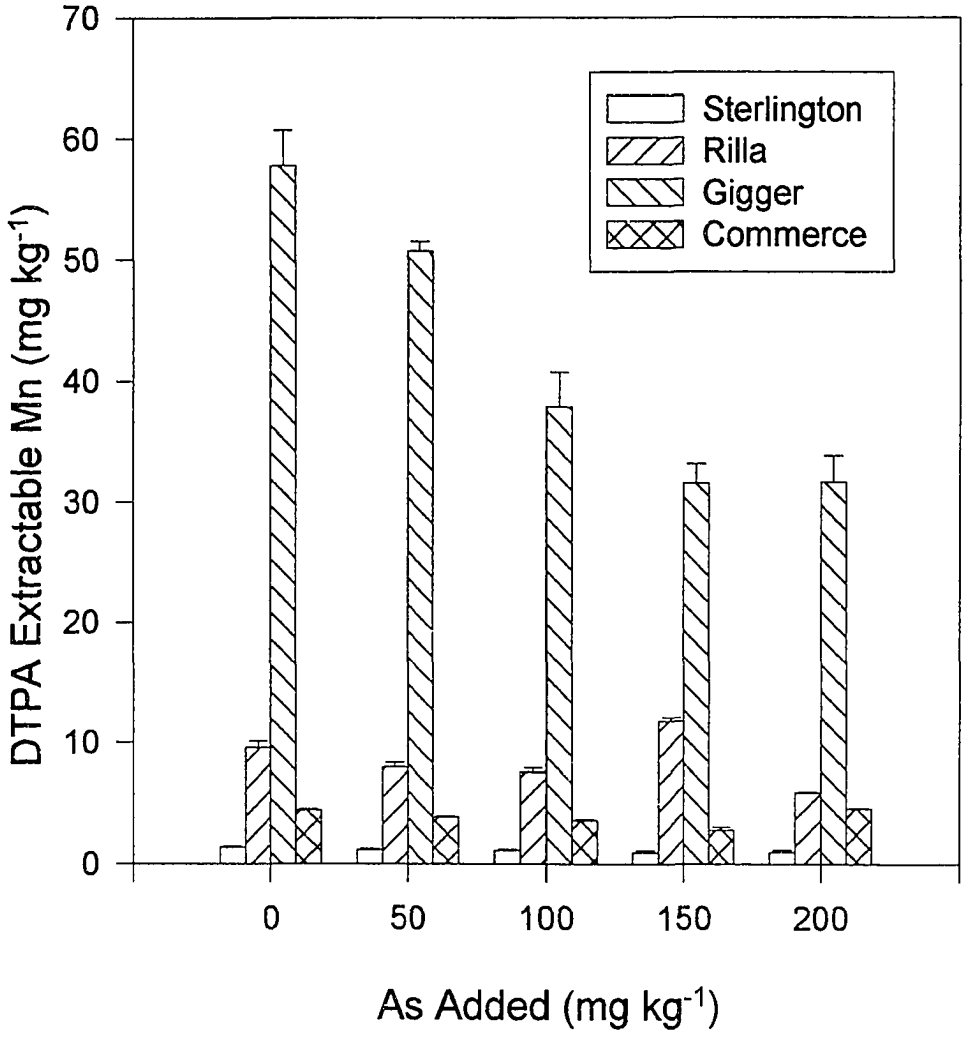

Figure 2.2 Effect of As addition on DTPA-extractable Mn for four soils. Error bars indicate significance at 0.05 level.

was found when the "c" values of the soils were compared to the initial solution As concentration as a percent of the total As in the soil.

The negative correlation between DTPA-extractable Mn and "c" indicates that this Mn fraction in the soil provides As adsorption or complexation sites, thus reducing the relative increase of solution As with As addition. Smaller increases in solution As concentrations result in relatively smaller "c" values and thus a flatter curve as with the Gigger soil (Figure 2.1). Therefore, small "c" values can have two interpretations: an increase in solution concentration where the entire relationship is linear, as observed with the Gigger soil, or an increase in solution concentration that occurs after an initial curvilinear phase, as observed with the Sterlington soil.

In addition to the "c" value, the "a" value in the equation can also be important (Kovar and Barber, 1988). This value represents the relative linear increase in solution As with As addition. As the "a" values of the soils increase, the relative solution As concentration increases (Figure 2.1). Compared with the other soils, the Sterlington soil has a relatively low "c" value, but a relatively high "a" value, so that more As remained in solution as arsenic was added. The exception to this was the Gigger soil. The "a" value of the Gigger soil was higher than those of the other three soils, yet the increase in solution As was significantly less. Therefore, the "c" value had more influence over the relationship between solution As and added As in the four soils used in this study. These results suggest that cotton-producing soils with low initial solution As and elevated DTPA-extractable Mn would not supply phytotoxic amounts of As to roots, even after further As addition.

When As is adsorbed, other anions on adsorption sites should be displaced into solution. Since As is specifically adsorbed, it can displace other specifically adsorbed anions, such as P. Therefore, solution P concentrations also were measured to determine the effect of As addition. As arsenic was added, solution P levels increased in all four soils. Solution P levels increased from 0.02 g P m^{-3} to 0.3 g P m^{-3} in the Commerce soil, 0.01 g P m^{-3} to 0.07 g P m^{-3} in the Gigger soil, 0.01 g P m^{-3} to 0.58 g P m^{-3} in the Rilla soil, and 0.12 g P m^{-3} to 1.65 g P m^{-3} in the Sterlington soil. These results indicate that As anions displaced P anions from the adsorption sites.

While As rates up to 200 mg kg^{-1} were necessary to describe the entire relation, amounts between 0 and 50 mg kg^{-1} are more representative of those commonly found in cotton soils (Ori et al., 1993). Based on data within this range provided by the nonlinear functions, a much greater proportion of the added As remained in solution with the Sterlington soil relative to the other soils (Figure 2.3). It is also interesting to note that solution concentration at any one As rate varied significantly among the soils. For instance, when 20 mg kg^{-1} As was added, the solution As predicted by the curve for the Commerce soil was less than half that predicted by the curve for the Sterlington soil, while predicted values for the Rilla and Gigger soils are nearly undetectable (Figure 2.3).

The curvilinearity of the solution As - As added relationship affects the relative proportion of added As that remains in solution when the four soils are compared (Figure 2.1 and Figure 2.3). When 50 mg As kg^{-1} were added to the soils, the solution As in the Commerce soil was greater than that in the Rilla soil. However, because the

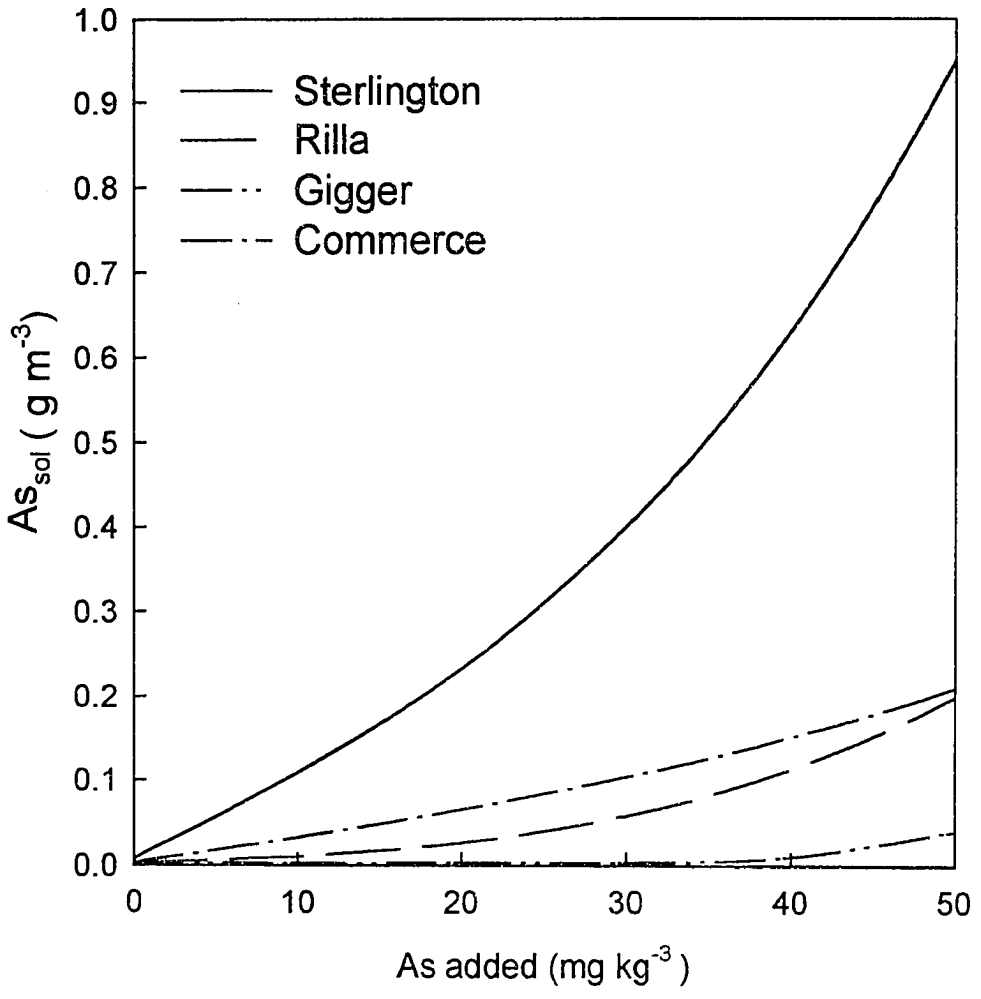

Figure 2.3 Predicted response of solution As to 0 to 50 mg kg^{-1} added As. Nonlinear regression was used to develop the relations.

relationship for the Rilla soil was more curvilinear, the solution As in the Rilla soil became greater than that in the Commerce soil at As rates greater than 50 mg kg^{-1}. Therefore, if less than 50 mg kg^{-1} of As is added to both soils, relatively more will remain bioavailable in the solution phase of the Commerce soil compared with the Rilla soil. If a significantly larger amount is added, relatively more would remain in solution in the Rilla soil. This suggests that the availability of As to plant roots varies not only with the amount of As applied, but also with the soil to which it is applied

Effect of As Addition on Resin-Exchangeable Solid-Phase As

The rate of increase in resin-exchangeable solid-phase As decreased curvilinearly with As addition for the Commerce, Rilla, and Sterlington soils (Figure 2.4) and could be described by the equation $As_{resp}=mx^{l}+n$, where As_{resp} is the resin-exchangeable solid-phase As concentration, x is the amount of As added, and m, l, and n are regression coefficients. The "m" values ranged from 0.14 to 13.42, "l" values ranged from 0.048 to 0.99 (for this relationship, smaller "l" values indicate greater curvilinearity), and "n" values ranged from 0.37 to 1.71 g m^{-3}. Values for regression coefficients for the individual soils are shown in Table 2.2. The curvilinear relationships show that the proportion of added As remaining in resin-exchangeable solid-phase form decreased with As addition. This suggests that the number of adsorption sites with resin-exchangeable As decreased as As was added to each soil. In the Gigger soil, however, the change in resin-exchangeable solid-phase As with As addition was nearly linear ("l"=0.986, a "l" value of 1 represents a straight line), implying a large number of adsorption sites in this soil.

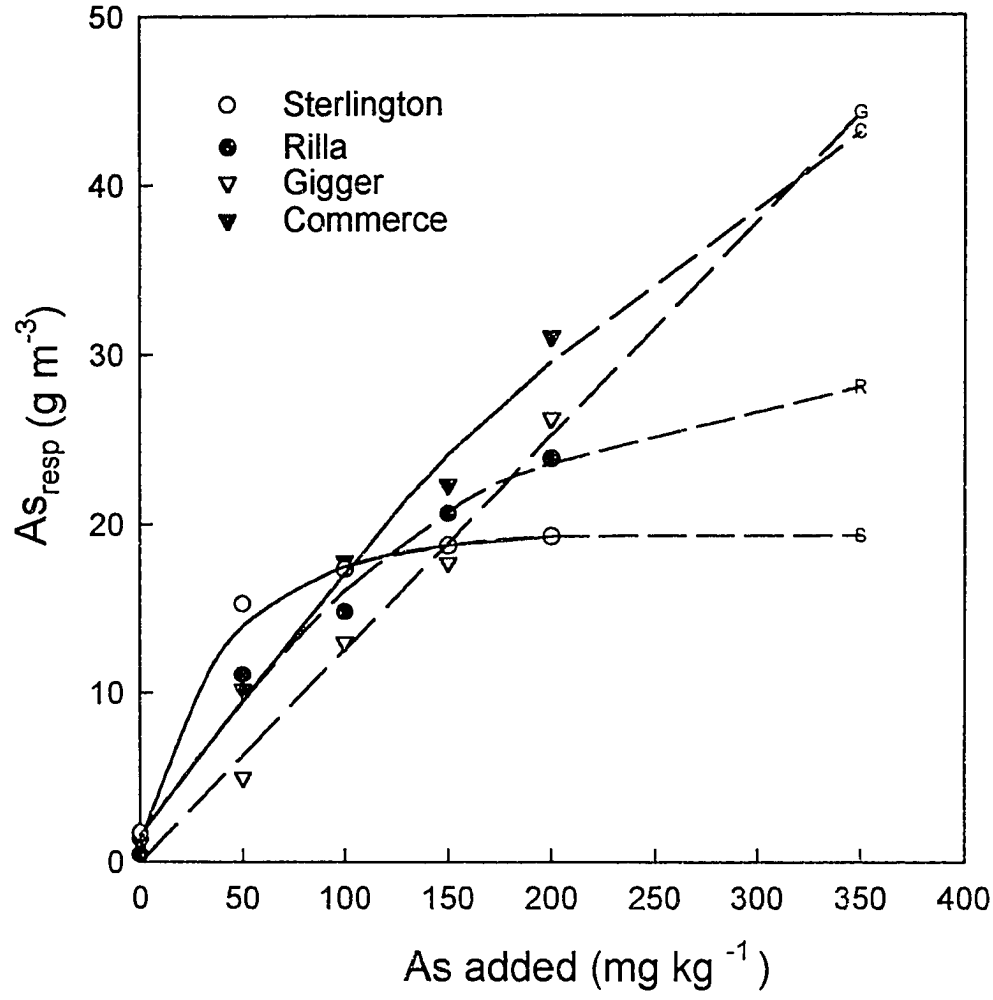

Figure 2.4 Relationships between resin-exchangeable solid-phase As and As added to four soils. Observed values fit the equation: $As_{resp}=m(As\ added)^{l}+n$. Dashed lines represent predicted resin-exchangeable solid phase As to 350 mg.

Initially, the Sterlington soil had the largest proportion of added As remaining in the resin-exchangeable solid phase, while the Gigger soil had the least proportion (Figure 2.4). However, as more As is added, the amount of resin-exchangeable solid phase As reached a maximum and the curve became flatter, indicating that the adsorption sites in the soil were saturated with resin-exchangeable As . Beyond this point, the added As remained in the solution phase or was adsorbed in non-resin-exchangeable form. However, an abundance of adsorption sites for resin-exchangeable As in the Gigger soil was indicated by the lack of curvilinearity in the relation. The dashed lines in Figure 2.4 represent the predicted resin-exchangeable solid-phase As concentrations from additions of 200 mg As kg^{-1} to 350 mg As kg^{-1}. These projections show that the Commerce, Rilla, and Sterlington soils had reached or were approaching the point where the adsorption sites in the soil were saturated, while the Gigger soil still readily adsorbed As in resin-exchangeable form. Comparisons of the nonlinear parameters with the soil properties yielded no correlations.

Relation Between Solution As and Total Diffusible As

The relation between total diffusible As and solution As represents the As buffer power of the soil over the concentration range and was curvilinear for all soils (Figure 2.5). Nonlinear regression was used to describe this relation for the four soils. Similar to the equation used by Kovar and Barber (1988), the equation $As_{td}=gAs_{sol}^{h}$ was used, where As_{td} represents the total diffusible As and g and h are regression constants. The values of "g" and "h" for the soils ranged from "g"= 15.08 to 55.11 and "h"= 0.28 to 0.88 (Table 2.2). The relation between total diffusible As and solution As shows that the

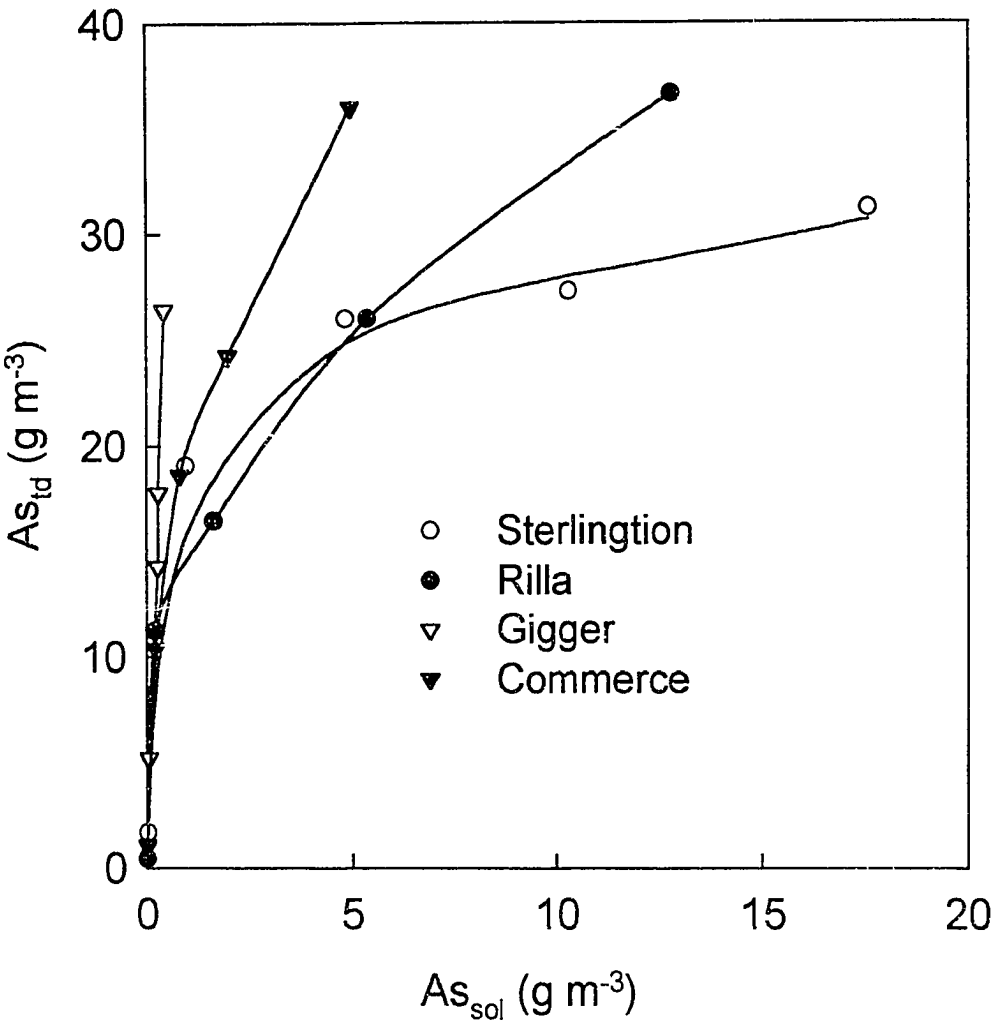

Figure 2.5 Relation between total diffusible As and As in soil solution. Observed values fit the equation: $As_{td}=g(As_{sol})^{h}$.

capacity of the total diffusible As to buffer changes in the solution As concentrations varied among the soils. For instance, at a solution concentration of 0.1 g m^{-3} As, the buffer power ($\delta As_{id}/\delta As_{sol}$) in the Sterlington soil was 23, while in Gigger soil it was 49. Hence, the Gigger soil can more readily maintain low As concentration in solution as As is removed by root absorption. Contrary to this, the relatively lower buffer power of the Sterlington soil indicates that solution As concentration would not be as readily maintained when As was removed from solution.

In general, at low levels of solution As, added As will be adsorbed or complexed in the soil, rather than remaining in the soil solution. As the number of As adsorption or complexation sites in the soil decreases, more added As remains in solution. Thus, soils with fewer adsorption or complexation sites such as the Sterlington and Rilla have less As retention on the solid phase and have a higher potential bioavailability of soil As. Soils such as the Gigger and Commerce silt loams have a larger affinity for the As, therefore the potential for As bioavailability is much less. For these four soils, the regression coefficients "g" and "h" were not correlated with any easily-measured physical or chemical properties.

Conclusions

Soil solution As and resin-exchangeable solid-phase As of the four soils used in this study responded differently to As addition. Soil solution As increased curvilinearly, while resin-exchangeable solid-phase As approached a maximum with As addition. Initial solution As and DTPA-extractable Mn were correlated with the response of the solution As concentration to As addition. Therefore, similar soils with large amounts of

DTPA-extractable Mn would have low levels of solution As after As addition. The change in resin-exchangeable solid-phase As after As addition approached a point in each soil where the adsorption sites in the soil became saturated and any additional As remained in solution. The relationships among solution As, resin-exchangeable solid-phase As, total diffusible As, and As addition can provide valuable information for use in mechanistic models that predict As bioavailability.

CHAPTER 3

EVALUATION OF A MECHANISTIC MODEL TO PREDICT ARSENIC UPTAKE BY CANOLA

Introduction

Arsenic is a naturally occurring element in nature and can be found in most environments. In virgin soils, total As concentrations average about 5 mg kg^{-1} and rarely exceed 10 mg kg^{-1} (Adriano, 1986). However, agricultural use of As can lead to increased concentrations in the soil. Woolsen et al. (1971) compared 58 surface soils with histories of As application to soils that had had no As applied. They found that the soils with As applied averaged 13-fold more As than the virgin soils (Woolsen et al., 1971). In Louisiana soils with histories of cotton production, the average total As concentration was 23 mg kg^{-1} (Ori et al., 1993) due mainly to the use of arsenical herbicides. Between the early 1900's and the 1960's, calcium arsenate was the major arsenical herbicide used in cotton production. Organic arsenicals (monosodium methane arsenate and disodium methane arsenate) appeared in the mid 1960's and replaced the inorganic arsenicals.

When applied to target species, As acts as a contact herbicide, thus root absorption is not important. However, the As returns to the soil as the plant residues decompose. It is this soil As that is potentially available for uptake by crop plants.

Cotton, an As-tolerant crop, is generally grown on highly-productive, well-drained soils. Although not common in Louisiana, rotations with other crops are feasible and beneficial in some cases. One possible crop for rotation with cotton is canola

(*Brassica napus* L.). Canola is an oilseed crop from which a high quality oil fit for human consumption is produced. Since canola oil is low in saturated fat, its demand is increasing as the health consciousness of the public increases. Canola grows best on well drained soils, thus this crop would fit well into a rotation with cotton. However, the presence of soil As may be a limitation to canola production. Arsenical herbicides can effectively control wild mustard (*Brassica kaber*), a relative of canola, suggesting that canola may be sensitive to soil As. (U.S.EPA, 1975). Hence, the effect of soil As on canola would be of interest in deciding whether to include canola in a rotation with cotton.

An effective way to investigate which soil or plant growth parameters control As uptake is through modeling. Mechanistic models now exist that can accurately predict ion uptake by plants. The model developed by Barber and Cushman (1981) combines mathematical descriptions of soil and plant processes to predict uptake. Various studies have shown that the model accurately predicted phosphorus (P) and potassium (K) uptake on a variety of crops grown on different soils (Barber, 1984). Since As and P ions are chemically similar, the model may also be able to predict As uptake. Values for 12 parameters are necessary to calculate uptake with the model.

In the Barber-Cushman model, soil supply of an ion by mass flow and diffusion is described with a transport equation (Nye and Tinker, 1977). Three parameters (D_e, the effective diffusion coefficient, b, the buffer power of the soil, and C_{li}, the initial soil solution concentration of the ion) are used in the transport equation to describe the soil supply characteristics of the ion. Values for these parameters depend on soil properties

and must be determined for individual soils. Changes in these parameters are independent of the plant.

Four parameters are used to describe root growth and morphology and are determined from harvested roots. Root growth and morphology characteristics are described by: L_o, the initial root length; r_o, the average root radius, r_1, the average half distance between roots, and k, the root growth rate.

Three parameters are used to describe the kinetics of ion uptake by the roots. Ion uptake kinetics are described by: I_{max}, the maximum ion influx rate, K_m, the ion solution concentration where influx is equal to 0.5 I_{max}, and C_{min}, the minimum solution concentration needed for ion uptake. These kinetic parameters are commonly determined with a depletion method similar to that developed by Claassen and Barber (1974). In this method, the depletion of the ion of interest from a solution containing actively growing roots is measured over a relatively short period of time, e.g. 24 hrs. The ion concentration in solution is then plotted against the time interval. The maximum rate of ion depletion represents I_{max}. The Michaelis-Menten constant, K_m, is found at 0.5 I_{max}. However, the toxic effects of As can slow or stop active uptake by the plant by uncoupling oxidative phosphorylation (Amburgey, 1967). Hence, the As toxicity effects may not be reflected in a short-term depletion study. Therefore, it is necessary to determine As uptake kinetics from plants grown for longer periods of time. In the method of Seeling and Claassen (1990), the equation of Baldwin et al. (1973) for diffusive transport is used to determine the ion concentration at the root surface (C_{lo}) and the Williams equation (1946) is used to determine ion influx (I_n) into the plant. The

uptake parameters I_{max} and K_m are estimated by conducting the appropriate kinetic analysis, e.g. Lineweaver-Burke Plot, Hanes plot etc. (Tinoco et al., 1985)

In order to determine which of the model parameters most influence As uptake, a sensitivity analysis can be used. In this analysis, an individual parameter is changed while all other parameters are held constant. This shows the relative effect of each parameter on predicted uptake. A limitation with this analysis is that it assumes that changing one parameter does not affect the other parameters. This is not always a valid assumption; however, this analysis can provide some insight as to which of the model parameters most influences ion uptake.

The purpose of this study was to determine if soil arsenic affected the growth of canola and which soil or plant parameters most influenced As uptake. The specific objectives of this research are to: I. determine the effect of added As on the growth of canola. ii. evaluate the ability of the Barber-Cushman mechanistic model to predict As uptake, and iii. determine which soil supply and root growth and morphology parameters are most influential in determining As uptake.

Materials and Methods

Three As rates (0, 5, and 10 mg kg^{-1}) were applied to three soils in a 3 by 3 factorial arrangement. The soils were: Commerce silt loam (fine-silty, mixed, nonacid, thermic, Aeric Fluvaquent), Rilla silt loam (fine-silty, mixed, thermic, Typic Haplaudalf), and Sterlington silt loam (coarse-silty, mixed, thermic, Typic Haplaudalf). Sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) was dissolved in deionized water, applied as solution, and thoroughly mixed with the soil. The soils were allowed to equilibrate for 30 days at -33

kPa moisture tension. After measuring the root length at planting, 4 14-day-old canola seedlings were transplanted into 3 L pots containing 2.5 kg (oven dry weight basis) of soil. The plants were grown for 14 days in a controlled climate chamber with a day temperature of 20°C and a night temperature of 15°C. Daylength was set to 16 hrs. Deionized water was added as needed to maintain the soil at -33 kPa moisture tension. The pots were covered with black film to reduce evaporative losses and pots without plants were used to measure what losses occurred.

After 14 days, shoot tissue was harvested at the soil level. Large roots were separated from the soil by hand and a soil subsample was shaken in water to separate the finer roots. Root length was determined from digitized images developed with a desktop scanner (Pan and Bolton, 1991). Shoot and root dry weights were measured. Shoots and roots were digested in concentrated HNO_3. Arsenic concentrations were measured by atomic absorption spectroscopy with hydride generation (Ganje and Rains, 1982).

The Barber-Cushman mechanistic model (Barber and Cushman, 1981) was used to predict As uptake. Values for 12 parameters describing soil supply of the ion, root growth and morphology of the plant roots, and the kinetics of ion uptake by the plant roots were determined.

The parameters C_{li} and b were calculated from nonlinear regression equations describing the As soil supply characteristics for these soils determined in an earlier experiment. In the earlier experiment, 5 rates of As from 0 to 200 mg kg^{-1} were added to 4 cotton-producing soils, three of which were used in this study. After 30 days of equilibration, the C_{li} was determined by column displacement (Adams, 1974). This

method accurately described the unaltered composition of the soil solution. A 500 g sample (oven dry weight) of the equilibrated soil was packed into a plexiglass column to a density of approximately 1.3 Mg m^{-3}. Filter paper was placed on the top of each column. Deionized water was added to each column at a rate of 4 mL h^{-1} until the soils reached "field capacity" water content. The samples were allowed to equilibrate for 24 h, then 40 mL of deionized water were added at a rate of 4 mL h^{-1}. The displaced solution was collected and filtered through a 0.20 μm filter. The solutions were analyzed by inductively coupled argon plasma spectroscopy (ICAP). If As concentrations were near the ICAP detection limit (0.03 μg g^{-1}), atomic absorption spectroscopy with hydride generation was used (Ganje and Rains, 1982). The relationship between C_{li} and As added was determined using nonlinear regression (SAS Inst., 1990). Anion-exchange resin was used to determine total diffusible As (C_{id}). A modified method of Amer et al. (1955) was used. A 0.5 g sample (oven dry weight basis) of the moist, equilibrated soil, 5.0 g of Dowex 1x8 Cl^- saturated exchange resin (dia. >0.425 mm), and 100 ml of deionized water were added to a 400 mL plastic bottle. The samples were shaken for 24 h to desorb As from the soil. The soil and resin were separated by washing the soil from the resin. The resin was then shaken with 50 mL of 1 M HCl for 6 h to desorb As from the resin. The solutions were filtered through a 0.45 μm filter. As before, As in the solutions was analyzed by ICAP. If As concentrations were near the ICAP detection limit (0.03 μg g^{-1}), atomic absorption spectroscopy was used (Ganje and Rains, 1982). The relationship between C_{id} and C_l was determined using nonlinear regression (SAS

Inst., 1990). The values for the parameter b were found by determining the $\delta C_{ti}/\delta C_{li}$ at the C_{li} of interest. The parameter D_e was calculated from the equation:

$$D_e=D_l\theta_v f/b$$

where D_l is the diffusivity of the ion in water, θ_v is the volumetric water content of the soil, and f is the tortousity constant ($1.6\theta_v$ -0.17). Values for these parameters are shown in Table 3.1

The root growth and morphology characteristics of the plant were determined from plants grown in each soil at each As rate. The value for k was calculated from the equation:

$$k=(\ln L_t-\ln L_o)/(t_t-t_o)$$

where L_t and L_o are the root lengths at t_t (harvest) and t_o (planting). Initial root length was determined at planting from digitized images developed with a desktop scanner (Pan and Bolton, 1991). The mean half distance between roots was determined from the equation:

$$r_l=[1/(L_v \pi)]^{½}$$

where L_v is the root length density per pot. Values for V_o, the water influx rate, were found by measuring the water loss per pot and the root surface area. Values for these parameters are shown in Table 3.2.

Values for I_{max} and K_m were determined with an influx method as described by Seeling and Claassen (1990). Values for these parameters were: I_{max}:, $1.47e^{-9}$ μmole cm^{-2} s^{-1}, K_m: $7.04e^{-4}$ μmole cm^{-3}, and C_{min}; $1.00e^{-10}$ μmole cm^{-3}. The C_{min} value represents the solution concentration below which ion efflux occurs. Since the initial As

Table 3.1 Soil parameters used to predict As uptake. The parameter values were determined from equations developed in a previous experiment studying the As soil supply characteristics of these soils.

Soil	As rate	D_e	b	C_{li}
	mg kg^{-1}	cm^2 s^{-1}		µmole cm^{-3}
		$x10^{-9}$		$x10^{-4}$
Commerce	0	2.31	117.61	1.47
	5	2.33	116.64	1.49
	10	2.49	109.39	16.60
Rilla	0	0.98	260.61	1.46
	5	1.04	246.90	1.60
	10	1.41	181.72	2.40
Sterlington	0	1.04	153.07	4.67
	5	2.37	67.65	14.90
	10	5.13	31.33	42.70

Table 3.2 Root growth and morphology parameters used to predict As uptake. Parameter values were calculated from measurements made from harvested roots in a controlled climate chamber study.

Soil	As rate	r_o	r_1	k	V_o
	mg kg^{-1}	cm	cm	cm s^{-1}	cm^3 cm^{-2} s^{-1}
		$x10^{-3}$	$x10^{-1}$	$x10^{-6}$	$x10^{-6}$
Commerce	0	8.94a*	4.69a	2.28a	9.12a
	5	8.37a	4.51a	2.39a	8.14a
	10	7.89a	4.67a	2.36a	8.76a
Rilla	0	7.69a	8.14a	1.35a	10.20a
	5	7.50a	8.15a	1.46a	10.30a
	10	6.72a	8.19a	1.59a	9.24a
Sterlington	0	8.05a	3.61a	2.74a	7.21a
	5	8.73a	2.94a	3.00a	6.70a
	10	7.75a	3.41a	2.78a	7.60a

* Values within soils followed by the same letter are not significantly different

concentration of the plant is below detectable limits, little or no As efflux occurs, hence this parameter was set to an arbitrary value.

Results and Discussion

Effect of Added As on Plant Growth

Arsenic addition appeared to have little affect on plant growth. Plant dry weight in the Commerce soil decreased with arsenic addition, however.this decrease was not significant (Table 3.3). Root length in this soil increased when 5 mg kg^{-1} As was added but did not change with further As addition. As with the plant dry weights in this soil, this increase was not significant.

In the Rilla soil, As had no affect on plant dry weight (Table 3.3). Root length increased with As addition but the increases were not significant. Plants grown in this soil were significantly smaller ($P \leq 0.05$) than those grown in the other two soils suggesting that other soil factors were influencing plant growth.

The plants grown in the Sterlington soil also showed no significant effects of the As addition (Table 3.3). Plant dry weight and root length increased when 5 mg kg^{-1} of As were added to the soil but decreased with further As addition.

While As appeared to have little or no effect on canola growth, As toxicity symptoms were present in all treatments. The toxicity symptoms included wilting, severe chlorosis, and purpling of lower leaves. This would suggest that, while the plant growth was not significantly affected by the As addition, As was having an effect on plant metabolism.

Table 3.3 Plant growth, tissue arsenic concentration, and tissue arsenic uptake characteristics for 28-day-old canola grown in a controlled climate chamber.

Soil	As Rate	Shoot Dry Weight	Root Dry Weight	Total Plant Dry Weight	Root Length	Shoot As conc..	Root As conc.	Total As conc.	Total As Uptake
	mg kg^{-1}	g	g	g	cm	µmole g^{-1} $x10^{-2}$	µmole g^{-1} $x10^{-2}$	µmole g^{-1} $x10^{-2}$	µmole $x10^{-2}$
Commerce	0	1.56a*	0.14a	1.70a	3780a	1.70a	2.23a	1.75a	2.90a
	5	1.28a	0.16a	1.45a	4587a	2.83b	3.29b	2.89b	4.10b
	10	0.96a	0.14a	1.10a	4584a	3.64c	4.40b	3.75c	4.10b
Rilla	0	0.49a	0.04a	0.53a	1232a	4.88a	3.09a	4.72a	2.89a
	5	0.44a	0.04a	0.48a	1405a	5.19a	3.35a	5.36a	2.55a
	10	0.56a	0.04a	0.60a	1691a	5.55a	3.11a	5.01a	2.98a
Sterlington	0	2.19a	0.26a	2.45ab	6894a	3.14a	5.20a	3.34a	8.34a
	5	2.90a	0.39a	3.29a	7533a	5.55ab	13.78b	6.57b	21.55b
	10	1.82a	0.24a	2.06b	6924a	7.45b	16.56b	8.54b	17.19b

*Values within soils and columns followed by the same letter are not significantly different

Effect of Added As on As Uptake and Tissue As Concentration

The effect of the added As on As uptake differed for each of the three soils. Addition of 5 mg kg^{-1} resulted in a significant ($P \leq 0.05$) increase in uptake by the canola in the Commerce soil (Table 3.3). The change in uptake was negligible, however, when As addition was increased from 5 to 10 mg As kg^{-1}. This would indicate that 10 mg As kg^{-1} was slowing plant metabolism enough to hinder As uptake. In this soil, the increase in the calculated C_{li} from 0 to 5 mg As kg^{-1} was negligible (Table 3.1) while the calculated C_{li} for the 10 mg As kg^{-1} was 11 times that of the 5 mg As kg^{-1} treatment. Hence, it would appear that the large increase in the solution As concentration slowed plant uptake of As to the point where it was no different than that in the 5 mg added As kg^{-1} treatment. A large increase in uptake occurred from 0 to 5 mg added As kg^{-1} while the calculated C_{li} only increased slightly. One possible explanation is that the r_o value decreased in the 5 mg kg^{-1} from 0 mg As kg^{-1}, however this decrease was not significant. This smaller root radius would provide more root surface area for As uptake. This appears to be a valid reason for the increase in uptake, since the other soil supply and root growth parameters for these treatments were similar (Table 3.1 and Table 3.2).

Arsenic uptake by plants grown in the Rilla soil did not change significantly with As addition. This is not surprising considering the lack of change in the calculated C_{li} with increasing rates of As (Table 3.1). Solution As concentrations only increased a total of 0.32 μmole cm^{-3} as As addition increased from 0 to 10 mg added As kg^{-1}. Since this increase is small and the other growth parameters similar (Table 3.1 and Table 3.2), there is little reason to expect differences in the uptake.

Arsenic uptake by the plants grown in the Sterlington soil significantly increased when 5 mg As kg^{-1} were added. However, there was no significant difference between the As uptake in the 5 mg added As kg^{-1} treatment and the 10 mg added As kg^{-1} treatment. Arsenic uptake in the 10 mg added As kg^{-1} was slightly less than that in the 5 mg As kg^{-1} treatment (Table 3.3). This slight reduction in uptake could be a dilution effect due to the reduction in plant dry weight in this treatment. Arsenic uptake was higher in the Sterlington soil than the other two soils. The C_{li} values for this soil were approximately 10-fold higher in the 5 and 10 mg added As kg^{-1} treatments than were found from the other two soils, thus providing more readily-available As for uptake.

While the effect of added As on total As uptake was different for each soil, plant As concentrations generally increased with increasing As (Table 3.3). Tissue As concentrations significantly ($P \leq 0.05$) increased with increasing As rates in the Commerce and Sterlington soils. In the Rilla soil however, tissue As concentrations increased in the 5 mg As kg^{-1} treatment, while tissue As concentrations slightly decreased with further As addition. In this soil, no differences were significant. The lack of significance between tissue As concentrations in this soil is probably due to the growth problems experienced by the plants in this soil. Plant dry weight for the treatments in this soil were significantly ($P \leq 0.05$) less than those for the other two soils.

Root As concentrations were greater than the shoot As concentrations in Commerce and Sterlington soils, indicating that As tended to remain in roots rather than being translocated to the shoots (Table 3.3). Evidence supporting root accumulation of As has been found by other researchers (Marcus-Wyner and Rains, 1982). However, the

opposite occurred in the Rilla soil (Table 3.3). In this soil, shoot As concentrations were higher than root As concentrations. Again, it is believed that the poor plant growth is responsible for this difference from the other soils. Since the plant dry weights for the treatments in this soil were substantially less than those for the treatments in the other two soils, the resulting As concentration would be greater in this soil due to a concentration effect.

Evaluation of the Model

A depletion study similar to that of Claassen and Barber (1974) is commonly used to determine the uptake kinetics of an ion. However, this method may not be adequate when describing As uptake. With this method, the depletion of an ion from a solution containing actively growing roots is measured over a relatively short period of time, usually 24 hrs. Two possible problems can affect As depletion from solution, thereby corrupting the data. The first involves the effect of As on the active ion uptake mechanism and the second is a result of the initial As solution concentration used to measure depletion. Active ion uptake depends on the breakdown of the adenosine triphosphate (ATP) complex to provide energy to move an ion from an area of low concentration to an area of high concentration. Arsenic can substitute for P in the ATP molecule resulting in an adenosine diphosphate (ADP)-As complex. The presence of the As ion blocks the phosphorylation of the ADP molecule, thus no energy transfer occurs (Dixon and Webb, 1958). Hence, in the presence of As, active ion uptake can be slowed over a period of time. This decreased uptake rate may not be apparent in short term depletion study. The second potential problem in using the depletion study involves the

initial As solution concentration used to measure the As influx. When this method is used for a nutrient, the initial solution concentration is large enough to assume that the active uptake sites are saturated, thus resulting in the maximum influx possible. However, if this approach is used with As, the active uptake sites may stop functioning due to the increased As toxicity, leading to erroneous values of I_{max} and K_m. Due to the range of solution As concentrations possible in the soil, the depletion method may not be a feasible method for determining As uptake kinetics.

A second method for determining uptake kinetics uses the actual plant uptake of As and the concentration of As at the root surface to determine the uptake kinetics over time (Seeling and Claassen, 1990). This method has the advantage of accounting for changes in I_{max} and K_m. It also allows determination of kinetic parameters for the actual soil As concentration range present. Hence, we used the method of Seeling and Claassen to determine the uptake kinetics parameters for the model. The appropriate linear transformation for the data was a Hanes plot (Lasch, 1987) (Figure 3.1). In this transformation, C_{lo}/I_n is plotted against C_{lo} and linear regression is applied to the data. The x-axis intercept represents $-K_m$ and the y-axis intercept represents K_m/I_{max}.

The data from Table 3.1, Table 3.2, and Figure 3.1 were used in the model to predict uptake. The uptake predictions from the model were compared with the data from Table 3.3. The observed and predicted As uptake values for the three soils were combined for regression analysis. This allowed the model to be evaluated over a range

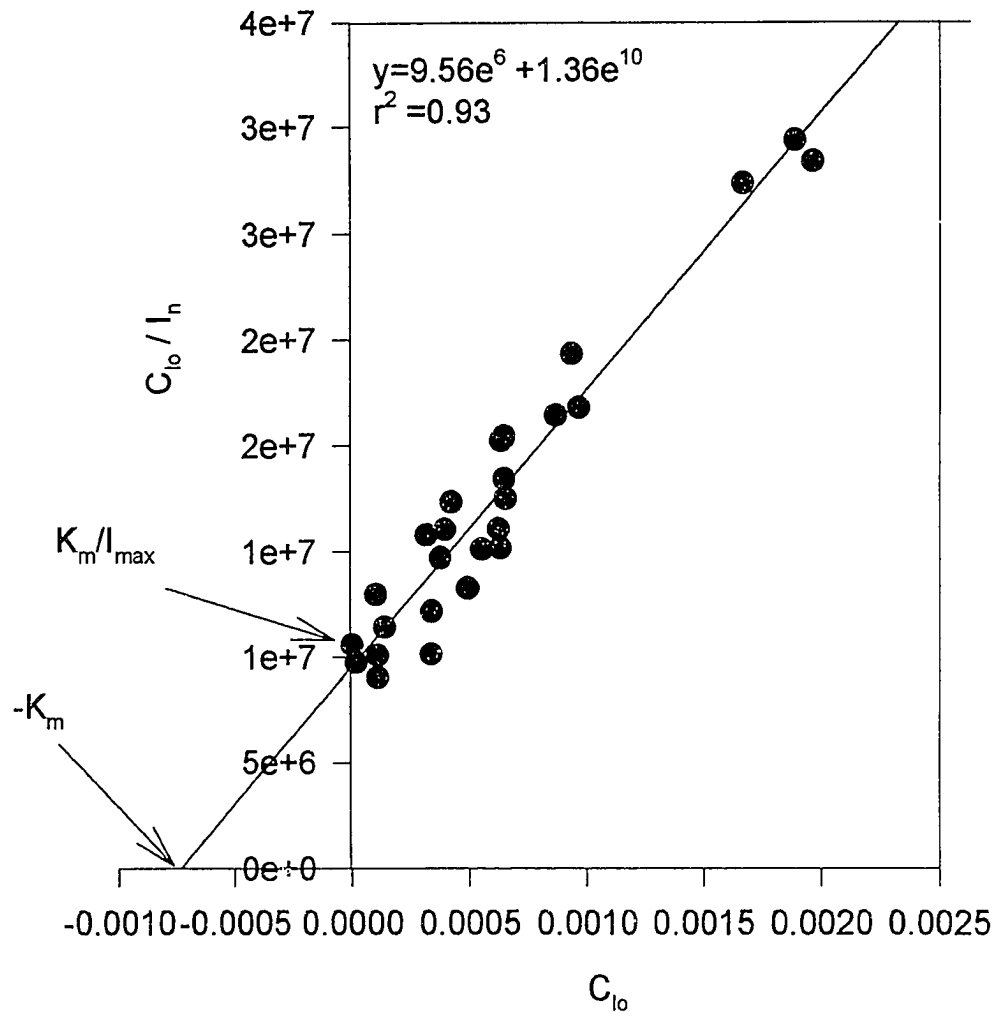

Figure 3.1 Hanes plot for describing I_{max} and K_m. The x-axis intercept represents $-K_m$ and the y-axis intercept represents K_m/I_{max}.

of soil conditions simultaneously rather than on a soil-by-soil basis. A good agreement was found between predicted and observed uptake. The relation between predicted (y) and observed (x) As uptake fit the equation $Y=5.11E^{-3}+1.01X$ ($r^2=0.96$, $P\leq0.05$) (Figure 3.2). The slope value of 1.01 indicates good agreement between predicted and observed As uptake by canola. Since the relatively higher As uptake in the Sterlington soil exerted a large influence on the regression function, a series of paired t-tests were conducted comparing the observed and predicted As uptake in each soil at each As rate. These t-tests found no significant differences ($\alpha=0.05$) between predicted and observed uptake within soils and rates, hence the model could be used to predict As uptake using these uptake parameters.

Sensitivity Analysis

A sensitivity analysis was performed to determine the relative effect of each parameter on As uptake. Arsenic uptake was calculated with the Barber-Cushman model when an individual parameter was multiplied by 0.5, 1, 1.5, and 2.0 of the measured value while all other parameters were held constant. Initial parameter values are shown in Table 3.4.

In all three soils, the influence of the parameters on As uptake were similar (Figure 3.3). The root growth rate, k, affected predicted As uptake the greatest (Figure 3.3). Assuming that no other parameters change, increasing the root length would provide more root surface area for absorption and thus increase As uptake over time. The parameter exerting the second greatest influence on predicted As uptake was the

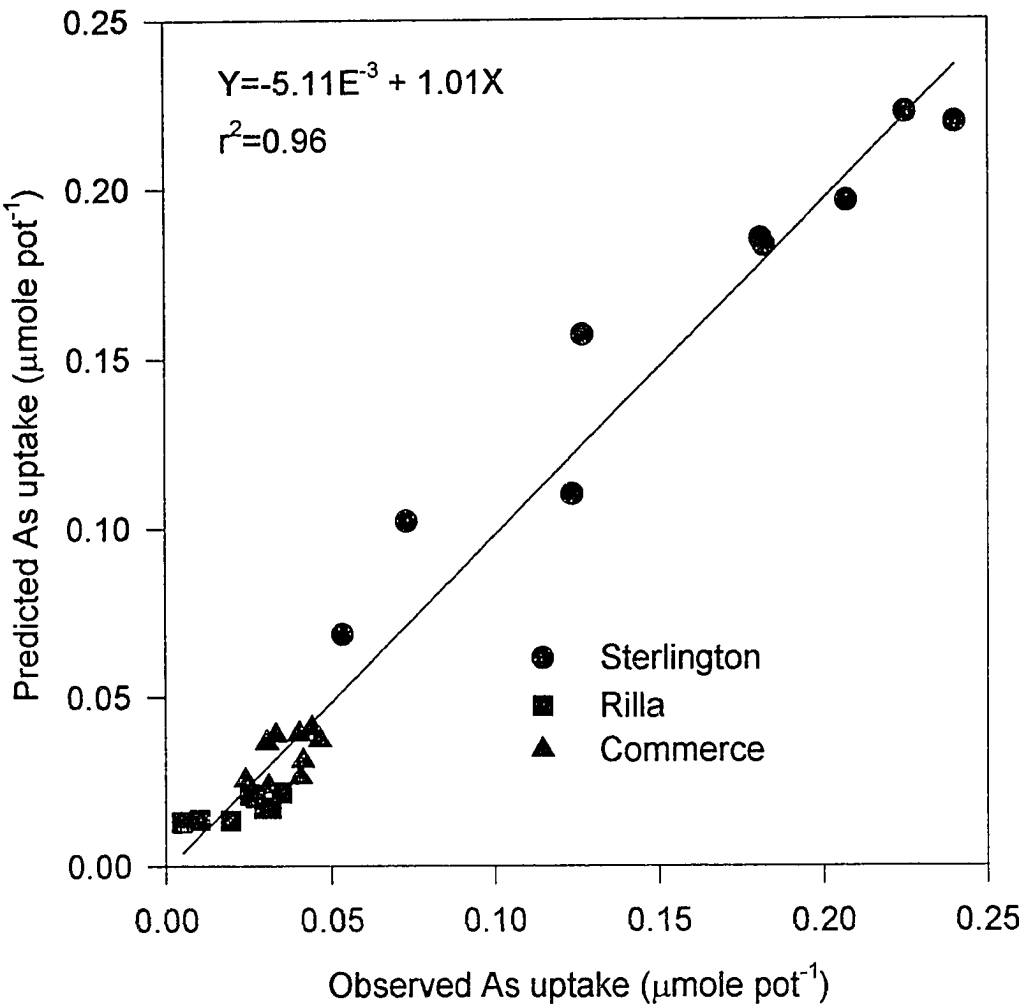

Figure 3.2 Predicted vs observed As uptake for 28-day-old canola grown in three soils in a controlled climate chamber. The slope value of 1.01 indicates good agreement between predicted and observed As uptake

Table 3.4 Initial parameters used in the sensitivity analysis. Arsenic uptake was predicted when each parameter was multiplied by 0.5, 1.0, 1.5, and 2.0 while all other parameters were held constant.

	Soil		
Parameter	Commerce	Rilla	Sterlington
D_e cm^2 s^{-1}	$2.31e^{-9}$	$9.83e^{-9}$	$1.04e^{-9}$
b unitless	117.61	260.61	153.07
C_{li} µmole cm^{-3}	$1.47e^{-4}$	$1.46e^{-4}$	$4.67e^{-4}$
V_o cm^3 cm^{-2} s^{-1}	$9.19e^{-6}$	$1.02e^{-5}$	$7.21e^{-6}$
r_o cm	$4.69e^{-1}$	$8.14e^{-1}$	$3.61e^{-1}$
r_1 cm	$8.94e^{-3}$	$7.69e^{-3}$	$8.05e^{-3}$
k cm s^{-1}	$2.28e^{-6}$	$1.35e^{-6}$	$2.74e^{-6}$
I_{max} µmole cm^{-2} s^{-1}	$1.47e^{-9}$	$1.47e^{-9}$	$1.47e^{-9}$
K_m µmole cm^{-3}	$7.01e^{-4}$	$7.01e^{-4}$	$7.01e^{-4}$
C_{min} µmole cm^{-3}	$1.00e^{-10}$	$1.00e^{-10}$	$1.00e^{-10}$

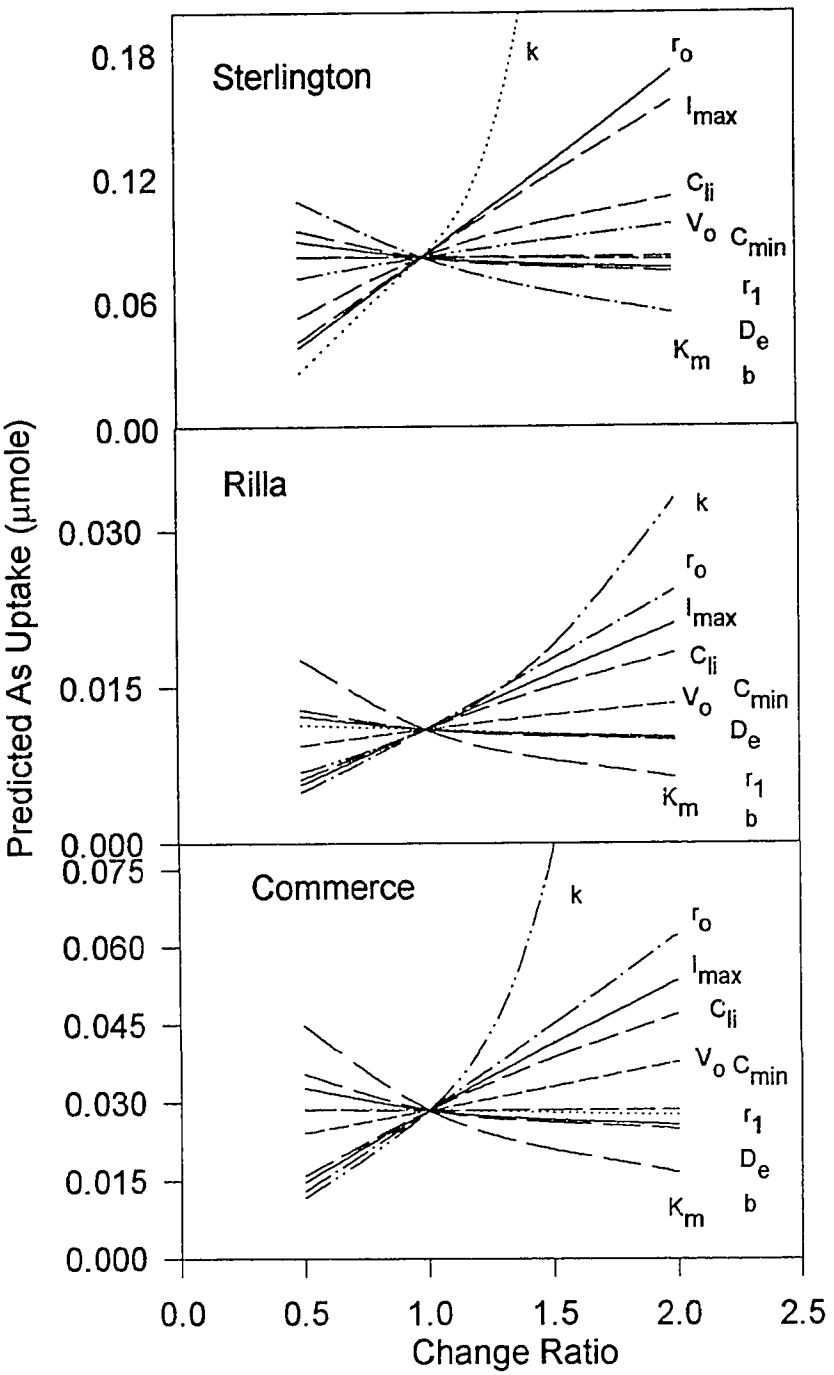

Figure 3.3 Sensitivity analysis where As uptake is predicted when each parameter is multiplied by 0.5, 1.0, 1.5, and 2.0 while all other parameters are held constant.

root radius, r_o. An increase in the root radius would also increase the root surface area for uptake. The next most influential parameter was I_{max}. This parameter represents the maximum influx possible, thus, an increase in I_{max} would infer more absorption sites in the roots and hence greater uptake. The initial As solution concentration followed I_{max} in the sensitivity analysis. An increase in C_{li} would provide more As at the root surface to be taken up. However, this can be misleading. By holding constant all the parameters except the one of interest, it assumes that changes in this one parameter will not affect the other parameters. For As, this is not a valid assumption. Increasing the C_{li} will be detrimental to root growth and affect uptake kinetics. This problem also holds true for the next parameter in the sensitivity analysis, V_o, the water influx rate. In the sensitivity analysis, an increase in V_o resulted in a higher level of As uptake. However, an increase in V_o in the soil would result in more As moving to the root, thus adversely affecting the root growth and morphology and uptake kinetics of the plant. Based on these results it would appear the As is moving to the root by mass flow but that As uptake is being controlled by the root processes involved with ion uptake.

Conclusions

In this study we have determined the effects of soil As on canola growth and evaluated a mechanistic uptake model with respect to As. We have also tried to determine what soil and plant parameters exert the most influence on As uptake. While As had no significant effects on plant growth, all treatments showed As toxicity symptoms. Hence, it would appear that plant metabolism is affected by As. Total As uptake by canola varied with soil type but seemed to be linked to the solution As

concentrations in the soils. Arsenic concentration in the plant significantly increased with increasing As rate, however As tended to remain in the root tissue as opposed to being readily translocated to the shoot tissue. The uptake model accurately predicted As uptake by canola. In a sensitivity analysis, the root growth rate and root radius exerted the greatest influence on As uptake, followed by the maximum uptake rate, I_{max}, and the initial soil solution As concentration. Root growth rate and root radius both affect the root surface area available for uptake. Hence, the decision to include canola in a rotation on a soil with a history of As application must be made on a soil by soil basis.

SUMMARY AND CONCLUSIONS

Arsenical compounds have been and are used as herbicides in cotton production in Louisiana. Hence, cotton production in Louisiana has led to increased soil arsenic concentrations in some soils. Currently there is little data available on the effect of arsenic addition on the different arsenic phases and on arsenic availability in the soil. There is also little data available on the effects of arsenic on canola, a possible new crop to Louisiana. Canola produces a high quality, edible oil that is becoming popular with consumers. The soils generally used for cotton production are ideal for canola production, hence a cotton-canola rotation may be favorable to producers wishing to increase efficiency. This study was initiated to study the reactions of the soil arsenic phases to arsenic addition and to determine the effects of arsenic on canola.

The first experiment studied the effect of arsenic form and concentration on canola growth and nutrient uptake. A solution study was used to test the effects of one inorganic arsenic form (arsenate) and two organic arsenic forms (MSMA and DSMA) at four different rates (0, 0.02, 0.5, and 1.0 mg As kg^{-1}). Fourteen-day-old canola was grown for 12 days in pots containing each form-concentration treatment. In the arsenate treatment, shoot and root arsenic concentration increased with solution arsenic concentrations to 0.50 mg As L^{-1}. At 1.00 mg As L^{-1}, shoot and root arsenic concentrations decreased. In the organic arsenic treatments, shoot arsenic concentrations tended to increase linearly with solution arsenic concentration. Root arsenic concentrations in the organic arsenic treatments also followed this trend. The organic arsenic treatments reduced shoot dry weights in the 0.50 and 1.00 mg As L^{-1}

treatments. All of the arsenic forms reduced the root length and root dry weight until the highest As rate where they increased. This increase was probably due to decreased ion uptake. As the plant nutrient demand exceeded nutrient uptake, the plant will increase root growth to increase nutrient uptake. This concept was supported by the root dry weight:shoot dry weight ratio. Shoot calcium and phosphorus tended to increase while zinc decreased with increasing solution concentrations in the organic arsenic treatments. Arsenate appeared to have no effect on the shoot nutrient concentrations. These results indicate that canola is sensitive to arsenic and arsenic form and concentration affect the toxicity. Inorganic arsenate appeared to reduce root growth while not showing any adverse effects on shoot tissue. The organic forms of arsenic affected both shoot and root growth while stimulating calcium and phosphorus uptake and depressing zinc uptake.

The second study was performed to determine the effect of arsenic addition on soil arsenic phases. Five arsenic rates (0, 50, 100, 150, and 200 mg As kg^{-1}) were added to four soils (Commerce silt loam, Gigger silt loam, Rilla silt loam, and Sterlington silt loam) and allowed to equilibrate. Total initial arsenic, arsenic in displaced soil solution, and resin-exchangeable solid-phase arsenic were determined for each treatment. Soil solution arsenic increased curvilinearly with arsenic addition for all soils. Curvilinearity was negatively correlated to initial solution arsenic and DTPA-extractable manganese concentration. DTPA-extractable manganese appeared to remove arsenic from the solution phase. The concentration of the resin-exchangeable solid-phase arsenic increased at a decreasing rate with arsenic addition indicating that the adsorption sites

for this phase were becoming saturated and more of the added arsenic was remaining in the solution phase. The relationship between the total diffusible arsenic and the soil solution arsenic was described by nonlinear regression and varied between soils.

The third experiment studied the effect of soil arsenic concentrations on canola growth and determined if arsenic uptake by canola could be predicted using a mechanistic model. Three rates of arsenic 0, 5, and 10 mg As kg^{-1} were added to three soils (Commerce silt loam, Rilla silt loam, and Sterlington silt loam) and allowed to equilibrate. Fourteen-day-old canola was grown in each treatment for 12 days then harvested. A mechanistic model accurately predicted arsenic uptake by canola. Root growth rate and root radius were found to have the most influence on arsenic uptake by canola. Canola appeared to be sensitive to soil arsenic in all of the treatments. Arsenic toxicity symptoms were present in each treatment. Total arsenic uptake by canola appeared to depend on the soil solution arsenic levels. Plant arsenic concentrations increased with arsenic rate and tended to remain in the plant roots.

The results of this study indicate that canola seedlings are sensitive to arsenic and that the form and concentration of the arsenic affect the toxicity. The results also indicate that arsenic addition affects the different phases of soil arsenic resulting in higher bioavailability of the arsenic. The higher bioavailability of the arsenic can lead to increased uptake by plants which can be predicted using a mechanistic model.

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Shoot ion concentrations of 28-day-old canola grown in a controlled climate chamber study in the As V, MSMA, and DSMA treatments.

		As Form								
Nutrient	Control		As V			MSMA			DSMA	
		0.02	0.50	1.00	0.02	0.50	1.00	0.02	0.05	1.00
		------	------	-------	------	mg L^{-1}	------	--------	-----	-----
B	75.44	76.80	76.25	76.97	73.78	71.78	72.10	74.54	66.15	73.55
Ca	26765	28265	25919	28147	29863	33144	34127	29447	31410	33020
Cu	2.90	4.12	3.47	4.36	2.53	2.67	2.91	2.99	2.75	2.47
Fe	71.11	80.88	74.39	91.37	75.36	85.86	79.32	98.21	70.33	71.89
K	41886	48964	50251	38778	48895	44315	48772	47662	44619	47738
Mg	3427	3835	3621	35.69	4043	3797	4361	3765	3778	4223
Mn	52.23	48.41	49.49	58.36	37.94	55.42	50.94	47.42	48.14	52.10
P	7542	8812	7492	8399	8621	11091	11124	7934	10353	12004
Zn	34.44	38.72	37.48	38.75	26.95	21.24	18.57	34.59	21.22	17.74

VITA

Michael Scott Cox was born October 22, 1965 in Indianapolis, Indiana to Kenneth L. and Rilda L. Cox. He graduated from Purdue University in December 1988 with a Bachelor of Science in Agronomy. After graduation, he accepted a graduate assistantship in soil fertility and plant nutrition under the direction of Dr. S.A. Barber at Purdue. After graduating from Purdue in May 1991, he married the former Lisa Kerkhof and accepted a graduate fellowship at Louisiana State University in Agronomy where he is presently a candidate for the degree of Doctor of Philosophy.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Michael Scott Cox

Major Field: Agronomy

Title of Dissertation: Arsenic Characterization in Soil and Arsenic Effects on Canola Growth

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

April 19, 1995